Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2907184 C 2022/12/06

(11)(21) 2 907 184

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) **Date de dépôt PCT/PCT Filing Date:** 2014/03/15
(87) **Date publication PCT/PCT Publication Date:** 2014/09/18
(45) **Date de délivrance/Issue Date:** 2022/12/06
(85) **Entrée phase nationale/National Entry:** 2015/09/15
(86) **N° demande PCT/PCT Application No.:** US 2014/029986
(87) **N° publication PCT/PCT Publication No.:** 2014/145254
(30) **Priorité/Priority:** 2013/03/15 (US61/790,153)

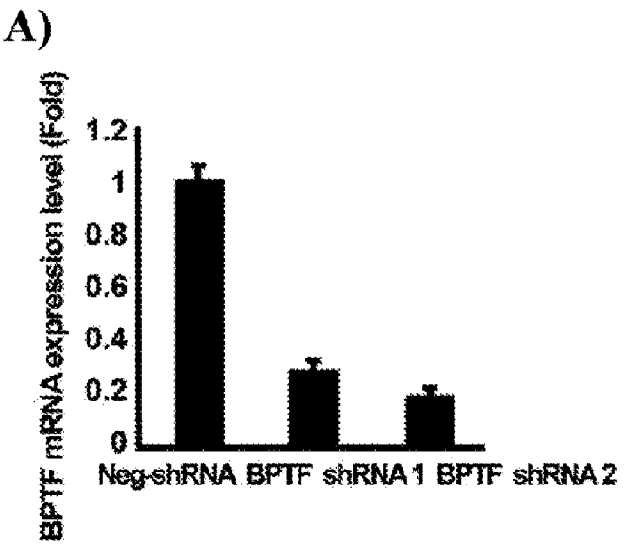
(51) **Cl.Int./Int.Cl.** ***G01N 33/48*** (2006.01),
A61K 45/00 (2006.01)

(72) **Inventeurs/Inventors:**
KASHANI-SABET, MOHAMMED, US;
DAR, ALTAF A., US

(73) **Propriétaire/Owner:**
SUTTER WEST BAY HOSPITALS, US

(74) **Agent:** MILLER THOMSON LLP

(54) **Titre : ANTIGENE FALZ DESTINE A ETRE UTILISE COMME CIBLE POUR THERAPIES DESTINEES A TRAITER LE CANCER**
(54) **Title: FALZ FOR USE AS A TARGET FOR THERAPIES TO TREAT CANCER**



(57) **Abrégé/Abstract:**
The disclosure provides methods for predicting and/or determining whether a subject has cancer based on the level of expression of BPTF. The disclosure also provides methods for determining whether a cancer in a subject is progressing or regressing based upon the change of expression levels of BPTF between two time points. The disclosure further provides methods treat a subject with a cancer by administering a polynucleotide comprising an inhibitory BPTF nucleic acid and/or an agent that inhibits the expression or activity of BPTF.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/145254 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01) *G01N 33/574* (2006.01)

(21) International Application Number:
PCT/US2014/029986

(22) International Filing Date:
15 March 2014 (15.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/790,153 15 March 2013 (15.03.2013) US

(71) Applicant: **SUTTER WEST BAY HOSPITALS** [US/US]; 475 Brannan Street, Suite 220, San Francisco, CA 94107 (US).

(72) Inventors: **KASHANI-SABET, Mohammed**; 153 25th Avenue, San Francisco, CA 94121 (US). **DAR, Altaf, A.**; 1490 Greenwood Way, San Bruno, CA 94066 (US).

(74) Agent: **BAKER, Joseph, R.**; Gavrilovich, Dodd & Lindsey LLP, 4660 La Jolla Village Drive, Suite 750, San Diego, CA 92122 (US).

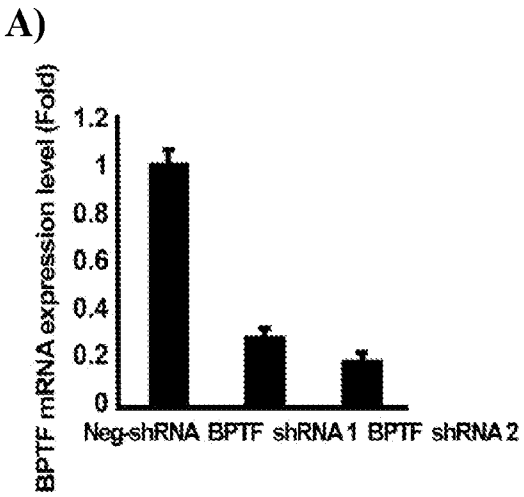
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

[Continued on next page]

(54) Title: FALZ FOR USE AS A TARGET FOR THERAPIES TO TREAT CANCER

(57) Abstract: The disclosure provides methods for predicting and/or determining whether a subject has cancer based on the level of expression of BPTF. The disclosure also provides methods for determining whether a cancer in a subject is progressing or regressing based upon the change of expression levels of BPTF between two time points. The disclosure further provides methods treat a subject with a cancer by administering a polynucleotide comprising an inhibitory BPTF nucleic acid and/or an agent that inhibits the expression or activity of BPTF.



WO 2014/145254 A3

WO 2014/145254 A3

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
18 December 2014

FALZ FOR USE AS A TARGET FOR THERAPIES TO TREAT CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119 from Provisional Application Serial No. 61/790,153, filed March 15, 2013.

GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with Government support under Grant Nos. CA114337 & CA122947, awarded by the National Institutes of Health and National Cancer Institute. The Government has certain rights in this invention.

TECHNICAL FIELD

[0003] The disclosure provides methods for cancer diagnosis, subject survival rate, and/or cancer progression based upon measuring the expression levels of FALZ. The disclosure further provides a method for treating a subject with cancer by inhibiting FALZ activity and/or expression.

BACKGROUND

[0004] Epigenetic mechanisms, including post-translational modifications of histones, DNA methylation, incorporation of histone variants, and nucleosome remodeling have evolved to regulate the structure of chromatin and access to DNA.

SUMMARY

[0005] BPTF (bromodomain PHD finger transcription factor; also referred to as FALZ (fetal Alzheimer antigen)), is a gene whose precise role in neoplastic transformation is unclear. The disclosure shows that FALZ expression is an independent prognostic marker for primary melanoma, and may represent a predictive biomarker of response to targeted therapy in melanoma. In addition, shRNA-mediated suppression of FALZ resulted in significantly decreased growth of melanoma, glioblastoma, and breast cancer cells *in vitro* and/or *in vivo*, suggesting the potential therapeutic utility of targeting FALZ in the therapy of melanoma as well as other solid tumors.

[0006] The disclosure provides a method of prognosis of cancer, comprising: (i) obtaining a biological sample from a subject; (ii) measuring the level of BPTF in the subject's sample; (iii)

Date Reçue/Date Received 2021-04-30

comparing the level of BPTF in the subject's sample with the mean level of BPTF from one or more control biological samples; (iv) providing a prognosis that the subject may have cancer based on having a lower (or higher) level for BPTF in comparison to the mean level of BPTF in the controls. In one embodiment, the cancer is selected from the group consisting of melanoma, breast cancer and brain cancer. In another embodiment, the subject's biological sample is from a tissue biopsy. In yet a another embodiment, the one or more control biological samples are from tissue biopsies of benign nevi. In a further embodiment, the control biological samples comprise samples from the subject. In still another embodiment, the control biological samples comprise samples not from the subject. In one embodiment, the disclosure uses a labeled antibody or nucleic acid fragment that specifically binds to a BPTF polypeptide or polynucleotide, respectively.

[0007] The disclosure provides a method of determining whether a subject has a cancer, comprising: (i) obtaining a biological sample from a subject; (ii) measuring the level of BPTF in the subject's sample; (iii) comparing the level of BPTF in the subject's sample with the mean level of BPTF from one or more control biological samples; and (iv) determining whether the subject has cancer based on having a significantly lower (or higher) level for BPTF in comparison to the mean levels for BPTF in the controls. In one embodiment, the cancer is selected from the group consisting of melanoma, breast cancer and brain cancer. In another embodiment, the subject's biological sample is from a tissue biopsy. In yet another embodiment, the one or more control biological samples are from tissue biopsies of benign nevi. In a further embodiment, the control biological samples comprise samples from the subject. In yet another embodiment, the control biological samples comprise samples not from the subject. In one embodiment, the disclosure uses a labeled antibody or nucleic acid fragment that specifically binds to a BPTF polypeptide or polynucleotide, respectively.

[0008] The disclosure provides a method of determining whether a cancer in a subject is progressing or in remission, comprising: (i) obtaining a biological sample from a subject at a first time

point; (ii) measuring the level of BPTF in the subject's sample from the first time point; (iii) obtaining a biological sample from a subject at a second time point; (iv) measuring the level of BPTF in the subject's sample from the second time point; (v) comparing the levels of BPTF from the first time point with the levels from the second time point; and (vi) determining whether a cancer is progressing or is in recovery based upon the change in levels of BPTF from the two time points, wherein an increase in BPTF levels between the first time point and the second time point indicates that the cancer is in remission, and wherein an increase in BPTF levels between the first time point and the second time point indicates the cancer is progressing. In one embodiment the cancer is selected from the group consisting of melanoma, breast cancer and brain cancer. In another embodiment, the biological samples are from tissue biopsies. In yet another embodiment, the method includes administering an anti-cancer therapeutic agent to the subject following obtaining the first sample. In yet another embodiment, the method includes administering a BPTF inhibitory agent to the subject if the BPTF levels increased. In one embodiment, the disclosure uses a labeled antibody or nucleic acid fragment that specifically binds to a BPTF polypeptide or polynucleotide, respectively.

[0009] The disclosure provides a method of providing a prognosis of the survival rate of a subject who has a cancer, comprising: (i) obtaining a biological sample from a subject at a first time point; (ii) measuring the level of BPTF in the subject's sample from the first time point; (iii) obtaining a biological sample from a subject at a second time point; (iv) measuring the level of BPTF in the subject's sample from the second time point; (v) comparing the levels of BPTF from the first time point with the levels from the second time point; and (vi) providing a prognosis of the subject's survival rate based upon the change in levels of BPTF from the two time points, wherein an increase in BPTF levels between the first time point and the second time point indicates a poor survival rate for the subject, and wherein a decrease in BPTF levels between the first time point and the second time point indicates a better survival rate for the subject. In one

embodiment, the cancer is selected from the group consisting of melanoma, breast cancer and brain cancer. In another embodiment, the biological samples are from tissue biopsies. In one embodiment, the disclosure uses a labeled antibody or nucleic acid fragment that specifically binds to a BPTF polypeptide or polynucleotide, respectively.

[0010] The disclosure also provides a method of treating a cancer in a subject, the method comprising: inhibiting the expression of BPTF by administering an effective amount of an inhibitory BPTF nucleic acid and/or an effective amount of an agent that inhibits the expression of BPTF. In one embodiment, the cancer is selected from the group consisting of melanoma, glioblastoma multiforme and breast cancer. In another embodiment, the inhibiting of the expression of BPTF results in inhibiting or preventing the proliferation or migration of cancer cells. In yet another embodiment, the method is used in combination with one or more additional anti-cancer therapeutic agents. In a further embodiment, the one or more additional therapeutic agents are selected from the group consisting of platinum analogs, alkylating agents, alkyl sulfonates, androgens, anti-adrenals, anti-androgens, antibiotics, anti-estrogens, aromatase inhibiting 4(5)-imidazoles, anti-metabolites, folic acid analogues, ethylenimines and methylamelamines, folic acid replenishers, nitrogen mustards, nitrosureas, purine analogs, pyrimidine analogs, topoisomerase inhibitors, thymidylate synthase inhibitors, anti-cancer antibodies, chemotherapeutics, de-methylation agents, and targeted therapeutic agents. In yet another embodiment, the method comprises administering a vector comprising an inhibitory BPTF nucleic acid to the subject. In yet a further embodiment, the vector comprises an expression vector. In still a further embodiment, the vector comprises a replication competent retroviral vector.

[0011] The disclosure also provides a composition comprising a BPTF inhibitor and a first-line anti-cancer therapeutic.

[0012] The disclosure provides for one or more embodiments set forth in the accompanying drawings and the description below.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0013] **Figure 1A-F** shows effects of shRNA-mediated suppression of Bptf expression on murine melanoma. A) Downregulation of Bptf expression at the mRNA level by two different shRNAs in B16-F10 murine melanoma cells. B) Suppression of cell proliferation following Bptf knockdown. C-D) *In vivo* tumor cell growth and metastatic lung tumor burden is significantly suppressed by Bptf knockdown. E-F) Suppression of CCND2 and BCL-XL expression at the mRNA and protein levels after Bptf knockdown.

[0014] **Figure 2A-G** shows the effects of suppression of BPTF expression on human melanoma. A) Suppression of BPTF expression at the mRNA level in 1205-Lu cells. B) BPTF suppression induces G0/G1 arrest and reduces the S-phase of the cell cycle. CD) Significant suppression of cellular proliferation following BPTF knockdown as determined by assays of cell survival and colony formation, respectively. E) BPTF knockdown induces apoptosis in melanoma cells. F-G) *In vivo* tumor cell growth and metastatic tumor burden is significantly suppressed by BPTF knockdown.

[0015] **Figure 3A-D** shows the effects of BPTF expression on sensitivity to DNase-I treament and on the ERK1/2 pathway. A-B) Suppression of BPTF expression enhanced DNase-I hypersensitivity to the promoter sequences of BCL-XL and BCL-2 in 1205-Lu and C8161.9 cells. C-D) Expression of CCND2, BCL-XL and BCL-2 at the mRNA level in two melanoma cell lines after BPTF suppression in 1205-Lu and C8161.9 cells. C-F) Western blot analyses showing expression of various proteins after BPTF suppression in 1205-Lu and C8161.9 cells.

[0016] **Figure 4A-D** shows BPTF levels in primary cutaneous melanoma. A-B) Kaplan-Meier analysis of DMFS and DSS in melanoma patients with highest BPTF expression levels (curve 1) *versus* all other patients (curve 2). C-D) Representative photomicrographs of FISH analysis showing low and high BPTF copy number in tissue samples.

[0017] **Figure 5A-H** shows the effects of modulation of BPTF expression on sensitivity to selective BRAF inhibitors. A-B) BPTF

knockdown sensitizes 1205-Lu melanoma cells to vemurafenib and dabrafenib. C-D) BPTF overexpression in 1205-Lu melanoma cells confers resistance to BRAF inhibitors. E) H&E staining of metastatic melanoma specimen following acquired resistance to vemurafenib. F) Immunohistochemical analysis of BPTF expression of sample in (E) showing heterogeneous pattern of BPTF staining. G) Pan-melanoma staining of specimen in (E). H) Fluorescence in situ hybridization (FISH) analysis of BPTF copy in the resistant specimen.

[0018] **Figure 6A-I** shows the effects of suppression of BPTF expression on human glioblastoma cells. A) shRNA-mediated suppression of BPTF at the mRNA level in U251 cells. B-C) Significant suppression of cellular proliferation following BPTF knockdown as determined by assays of cell survival and colony formation, respectively. D) BPTF suppression induces G0/G1 arrest and reduces the S-phase of the cell cycle. E) BPTF knockdown induces apoptosis in U251 cells. F) mRNA expression of different genes following BPTF suppression. G) Western blot analysis showing expression of various proteins. H) *In vivo* growth of U251 cells in nude mice following BPTF suppression. I) BPTF expression in mesenchymal and proneural GBM subtypes.

[0019] **Figure 7A-C** shows BPTF suppression suppresses the invasive potential of B16-F10 murine melanoma (A), 1205-Lu human melanoma (B), and U251 human GBM (C) cells.

[0020] **Figure 8A-F** shows the effects of BPTF suppression on C8161.9 human melanoma cells. A) Suppression of BPTF at the mRNA level in C8161.9 melanoma cells. B-C) Significant suppression of cellular proliferation following BPTF knockdown as determined by assays of cell survival and colony formation, respectively. D) BPTF knockdown induces apoptosis in C8161.9 melanoma cells. E) BPTF knockdown suppresses the invasiveness of C8161.9 melanoma cells. F) *In vivo* tumor growth of C8161.9 cells is significantly suppressed by BPTF knockdown.

[0021] **Figure 9** shows the overexpression of BCL-XL or ERK rescues the effects of BPTF suppression on 1205-Lu melanoma cell survival.

[0022] **Figure 10A-C:** A) BPTF overexpression enhances cellular proliferation in 1205-Lu melanoma cell lines. B) Levels of expression of various genes following BPTF overexpression. C) Western blot showing expression of various proteins following BPTF overexpression.

[0023] **Figure 11A-B** shows Immunohistochemical analysis of BPTF expression in a tissue microarray showing illustrative photomicrographs of BPTF expression. A) Primary melanoma expressing low levels of BPTF; B) primary melanoma expressing high levels of BPTF.

[0024] **Figure 12A-B:** A) BPTF knockdown sensitizes LOX melanoma cell lines to vemurafenib treatment. B) BPTF overexpression in LOX melanoma cell lines confers resistance to vemurafenib treatment.

[0025] **Figure 13A=D:** A) H&E staining of metastatic tumor sample following resistance to dabrafenib. B) Immunohistochemical staining of tumor sample showing heterogeneous pattern of BPTF staining. C) Pan melanoma staining of metastatic melanoma. D) Fluorescence in situ hybridization (FISH) analysis of BPTF copy number in metastatic melanoma.

[0026] **Figure 14A-F:** A) shRNA-mediated suppression of BPTF mRNA in LN18 human glioblastoma cells. B-C) Suppression of cellular proliferation following BPTF knockdown as determined by assays of cell survival and colony formation, respectively. D) BPTF knockdown suppresses invasiveness of LN18 cells. E-F) Level expression of various genes following BPTF suppression. F) Western blot analysis showing expression of various proteins following BPTF suppression.

[0027] **Figure 15** shows that shRNA mediated suppression of FALZ in MDA-231 breast cancer cell lines suppresses tumor cell proliferation (left panel) and invasiveness (right panel).

[0028] **Figure 16** shows that shRNA-mediated suppression of FALZ suppresses colony formation ability (Left panel) and induces apoptosis (right panel) in MDA-231 breast cancer cell lines.

[0029] **Figure 17** shows FALZ knockdown in MDA-231 breast cancer cell lines suppresses *in vivo* tumor growth in nude mice (Left panel). The right panel shows FISH analysis on human breast cancer tissue specimens indicating increase in FALZ copy number.

DETAILED DESCRIPTION

[0030] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides and reference to "the cell" includes reference to one or more cells, and so forth.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

[0032] Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting.

[0033] It is to be further understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of."

[0034] The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure. Moreover, with respect to any term that is presented in one or more publications that is similar to, or identical with, a term that has been expressly defined in this disclosure, the definition of the term as expressly provided in this disclosure will control in all respects.

[0035] Nucleosome remodeling and the incorporation of histone variants are largely accomplished through the action of ATP-dependent chromatin-remodeling complexes, which represent critical components of the machinery that controls gene expression. ATP-dependent chromatin-remodeling factors are classified into four

major subfamilies (ISWI, SWI/SNF, CHD and INO80) based upon sequence homology of the associated ATPase.

[0036] Chromatin-remodeling factors have been recognized to play an increasingly important role in tumorigenesis given the recent demonstration of mutations in chromatin regulators in various human cancers. In addition, the 17q24 locus has long been presumed to contain oncogenes, given its amplification in a number of tumors. Bromodomain PHD finger transcription factor (BPTF; also referred to as FALZ) (whose gene resides on 17q24), the largest component of the NURF complex, has been implicated in embryonic development, thymocyte maturation, and chromatin remodeling. The NURF complex in mammals is a well-characterized ATP-dependent chromatin-remodeling complex. However, little is known about the functional role played by BPTF in tumorigenesis.

[0037] Nucleosome remodeling factor (NURF), identified in *Drosophila melanogaster*, is a key member of the ISWI family of ATP-dependent chromatin-remodeling factors. In mammals, BPTF (bromodomain PHD finger transcription factor) represents the orthologue of the *Drosophila* NURF301, the largest subunit of the NURF chromatin-remodeling complex. The NURF301 homolog exists across all eukaryotic species and appears to be evolutionarily conserved. NURF301 participates in the regulation of expression of engrailed 1 and 2, presumably by changing the periodic alignment of nucleosomes.

[0038] The NURF complex mediates some of its cellular functions through interaction with sequence-specific transcription factors. In *Drosophila*, heat shock factor (HSF), GAGA, and the artificial domain VP16 have been shown to interact with multiple surfaces on NURF301 and weakly with ISWI. NURF301 has two well-characterized domains that bind specific histone post-translational modifications. The PHD finger juxtaposed to the bromodomain interacts with H3K4me2/3 and the adjacent bromodomain binds H4K16ac. In addition, NURF likely interacts directly with DNA in a sequence-specific fashion. BPTF (also referred to as FALZ) has been reported to be essential in embryonic development and involved in ATP-dependent chromatin remodeling.

[0039] The disclosure demonstrates that BPTF (sometimes referred to as FALZ herein) is significantly overexpressed in metastatic melanomas and breast cancer by cDNA microarray analysis and FISH. The human BPTF gene (SEQ ID NO:1) is located on chromosome 17q24, which is a hotspot for chromosomal changes in many tumors. Amplification of 17q24 has been shown in breast cancer, and increased 17q24 copy number has been observed in other solid tumors. A translocation occurring at the 17q24.3 locus encompassing the BPTF gene was demonstrated in lung embryonic cells. FAC1 (Fetal Alz-50-reactive clone 1) a truncated form of BPTF, is upregulated in neurodegenerative diseases, exhibits sequence-specific DNA binding activity, and may function in transcriptional regulation. While the importance of remodeling complexes such as NURF is well understood, to date, the functional role of BPTF in tumorigenesis has been incompletely characterized.

[0040] This disclosure provides a functional and biological role of BPTF in melanoma, glioblastoma multiforme (GBM), and breast cancer. Although these cancer types were specifically analyzed, the role of BPTF in other tumors and cancers is contemplated. The disclosure demonstrates that targeted suppression of BPTF suppresses melanoma, GBM, and breast cancer cell proliferation; that BPTF copy number is elevated in a significant proportion of melanomas and breast cancers, and BPTF overexpression is a marker of reduced survival in human melanoma patients. Furthermore, BPTF modulates the ERK pathway and confers acquired resistance to selective BRAF inhibitors in BRAF-mutant melanoma cells.

[0041] The disclosure demonstrates the functional and biological significance of BPTF in tumor progression, with a specific focus on melanoma, glioblastoma, and breast cancer. Suppression of BPTF expression in murine melanoma cells and breast cancer cells resulted in significant suppression of tumor cell proliferation and invasiveness. *In vivo* studies confirmed the powerful role played by BPTF in melanoma progression, as a significant decrease in tumor cell growth and metastatic tumor count was observed by using different shRNAs targeting BPTF. The effects of BPTF on cell cycle progression and on *in vitro* and *in*

vivo tumor cell growth were confirmed in two human melanoma and GBM cell lines as well as in breast cancer cells.

[0042] To gain mechanistic insight into BPTF function in melanoma cells, integrated analyses identified the downregulation of BCL2, BCL-XL and CCND2, which are key mediators of tumor cell proliferation, cell cycle progression, and apoptosis, following suppression of BPTF expression. The downregulation of these genes was confirmed by qRT-PCR and western blot analyses in melanoma and GBM cell lines. As the NURF complex in mammals has been shown to influence nucleosome positioning and nuclease hypersensitivity sites *in vitro*, the disclosure demonstrates that suppression of BPTF expression enhanced sensitivity to DNase I treatment of the BCL2 and BCL-XL genes.

[0043] Beyond the mechanistic analysis of BPTF action in melanoma, breast cancer and GBM, a detailed analysis of the role of BPTF as a biomarker of melanoma was also performed. FISH analysis of primary melanoma revealed elevated BPTF copy number in 36% of cases, in contrast with benign nevi in which increased BPTF copy number was absent. This suggests the potential utility of BPTF as a potential diagnostic marker to distinguish melanoma from nevi. Furthermore, the increased BPTF copy number observed may provide a mechanism for BPTF activation in melanoma, identifying a genetic basis for the overexpression of BPTF observed in the gene expression profiling analysis. In addition, digital imaging analysis of BPTF immunostaining identified BPTF overexpression as an independent predictor of distant metastasis-free and disease specific survival in human melanoma patients. Thus, these studies showed that BPTF is both a predictor and a promoter of distant metastasis, the lethal event in melanoma progression. Taken together, these findings establish BPTF as a novel target, molecular marker and mediator of melanoma tumorigenesis and tumor progression. In addition, analysis of BPTF expression in GBM tissues and breast cancer cells indicated that BPTF expression was enriched in the pro-neural subtype of GBM, which responds poorly to chemotherapeutic agents and in breast cancer cells.

[0044] BRAF is a major oncogenic driver in melanoma by virtue of point mutations in 40-50% of cases. Mutant BRAF constitutively

activates the MAP kinase pathway, and transduces pro-proliferative and pro-survival signals in melanoma cells, in addition to promoting tumor cell invasion and angiogenesis. Importantly, suppression of BPTF expression produced pronounced anti-tumor effects in melanoma cells harboring either mutant (1205-Lu) or wild type (C8161.9) BRAF, and resulted in significantly reduced levels of phosphorylated ERK1/2 (Thr204/Tyr204) as well as its direct target p90RSK (Ser380), which represent downstream effectors of the MAPK pathway. Intriguingly, three of the genes that were suppressed upon BPTF knockdown (BCL2, BCL-XL and CCND2) are regulated by ERK. These findings provide evidence that BPTF activates the MAPK pathway and thereby enhances tumor cell proliferation, invasiveness and survival while suppressing tumor cell apoptosis.

[0045] Treatment of metastatic melanoma patients harboring a BRAF mutation with selective BRAF inhibitors has been shown to confer an overall survival advantage. However, complete responses are rare and acquired resistance to these agents develops in the majority of treated cases. Several mechanisms of acquired resistance have been described, primarily including reactivation of the MAPK pathway [via N-RAS mutations or COT/MAP3K8 kinase overexpression], activation of platelet-derived growth factor receptor β or via activation of the PI3K pathway (via overexpression of IGF1R. The disclosure shows that modulation of BPTF expression (by either overexpression or shRNA-mediated downregulation) significantly modulated sensitivity of mutant BRAF melanoma cells to selective BRAF inhibitors and further provides new evidence that chromatin-remodeling factors are involved in promoting acquired resistance to targeted therapies in cancer. In addition to the mechanistic analysis of sensitivity to BRAF inhibitors in melanoma cell lines, analysis of specimens prior to and following progression with selective BRAF inhibitors indicated increased BPTF copy number upon therapeutic resistance in a subset of cases. This suggests that BPTF activation can be selected for during resistance to targeted therapy in melanoma, given that it can promote melanoma cell proliferation and survival.

[0046] Surprisingly, there was a significant difference in the immunohistochemical analysis of BPTF expression in the pre-

treatment and progressing lesions. While BPTF expression in the pre-treatment metastatic tumor was homogeneous, analysis of the progressing lesions identified, in some tumors, distinct clones of cells with divergent morphology and BPTF expression. One clone of tumor cells (devoid of BPTF staining) represented apoptotic cells that appeared to have responded to therapy, while the other clone identified surviving cells (with prominent BPTF staining) that possibly represented the resistant clone. This leads to the intriguing speculation that the metastatic tumor resistant to targeted therapy is not homogeneously resistant, but rather composed of cell subpopulations that are still actively responding to treatment. These observations are consistent with the recent demonstration of intratumor molecular heterogeneity of renal cell carcinoma, and are supported by a recent study that showed heterogeneous immunohistochemical marker expression in metastatic melanoma following progression on targeted therapy. Taken together, these results clearly support efforts at examining the potential utility of combinatorial therapies that are planned or under way targeting some of the aforementioned mechanisms of resistance. In addition, they suggest BPTF targeting as a possible approach to overcome resistance to BRAF inhibitors, or as a possible combinatorial treatment for metastatic melanomas with mutant BRAF.

[0047] In a recent study, BPTF was among a group of chromatin-remodeling factors mutated in liver cancers by whole-genome sequencing. Analysis of the COSMIC database reveals mutations in BPTF in a small number of skin cancers, including melanoma. The disclosure demonstrates an oncogenic role for BPTF in melanoma, breast cancer and glioblastoma, driven in part by increased copy number in a subset of cases. The results presented herein assign a novel functional role for BPTF in tumor progression by virtue of its effects on tumor cell proliferation and survival, via activation of BCL2, BCL-XL, CCND2, and ERK. BPTF overexpression is an independent predictor of survival associated with melanoma. Further, increased levels of BPTF promote resistance to therapies targeting mutant BRAF and may be selected for during acquired resistance to these agents.

[0048] In view of the data provided herein and the examples below, the disclosure provides methods and compositions useful for (a) treating cancers having an aberrant expression of BPTF, (b) increasing targeted therapy using existing first line chemotherapeutics and antibodies by inhibiting induced drug resistance resulting from BPTF expression, and (c) diagnostics useful for identifying progression and therapy of cancer. The methods of treatment described herein can use inhibitor nucleic acid therapy (*e.g.*, shRNA, siRNA, antisense molecules and the like) to downregulate BPTF expression. Alternatively, the disclosure can improve a therapy by combining a BPTF inhibitor (*e.g.*, a bromodomain inhibitor) with first-line therapeutics. Exemplary bromodomain inhibitors are described in, *e.g.*, U.S. Pat. Publ. No. 2014/0066410.

In this latter embodiment, the therapeutic method and compositions would be a combination of a bromodomain inhibitor and a first-line therapeutic for the cancer. The bromodomain inhibitor can be administered prior to, in combination with, or after administration of a first-line therapeutic/chemotherapeutic.

[0049] As used herein, the term "antisense oligonucleotide" refers to an unmodified or modified nucleic acid having a nucleotide sequence complementary to a BPTF polynucleotide sequence including polynucleotide sequences associated with the transcription or translation of BPTF (*e.g.*, a promoter of a BPTF polynucleotide), where the antisense polynucleotide is capable of hybridizing to a BPTF polynucleotide sequence. Of particular interest are antisense polynucleotides capable of inhibiting transcription and/or translation of BPTF polypeptide-encoding polynucleotide either *in vitro* or *in vivo*. Such antisense oligonucleotides can be delivered to a target cell through gene therapy (*e.g.*, recombinant viral vectors), operably linked to charge neutralizing moieties (*e.g.*, TAT or other protein transduction domains, see, *e.g.*, US Pat. Publ. Nos.2009/0093425, 2009/0093026, and 2006/0222657)

or direct nucleic acid delivery techniques.

Date Reçue/Date Received 2021-04-30

[0050] As used herein; the terms "siRNA oligonucleotides", "RNAi oligonucleotides", "short interfering RNA", or "siRNA" are used interchangeably and refer to oligonucleotides that work through post-transcriptional gene silencing, also known as RNA interference (RNAi). The terms refer to a double stranded nucleic acid molecule capable of RNA interference "RNAi", (see Kreutzer *et al.*, WO 00/44895; Zernicka-Goetz *et al.* WO 01/36646; Fire, WO 99/32619; Mello and Fire, WO 01/29058). SiRNA molecules are generally RNA molecules but further encompass chemically modified nucleotides and non-nucleotides. siRNA gene-targeting experiments have been carried out by transient siRNA transfer into cells (achieved by such classic methods as liposome-mediated transfection, electroporation, or microinjection). Molecules of siRNA are 21- to 23-nucleotide RNAs, with characteristic 2- to 3-nucleotide 3'-overhanging ends resembling the RNase III processing products of long double-stranded RNAs (dsRNAs) that normally initiate RNAi.

[0051] Effective exploitation of the siRNA pathway to mediate gene silencing depends, in part, on efficient methods of intracellular delivery of siRNA. siRNA molecules tend to be short-lived in the cell, not readily deliverable to cell types that are difficult to transfect and relatively expensive to produce via chemical syntheses. (Jacks *et al.*, (2005) Biotechniques 39: 215-224; Bernards *et al.*, (2006) Nature Methods 3: 701-706).

[0052] One method for efficient intracellular delivery of siRNA is the use of short hairpin RNAs, or "shRNAs". shRNAs are single stranded RNA molecules that include two complementary sequences joined by a non-complementary region. *In vivo*, the complementary sequences anneal to create a double-stranded helix with an unpaired loop at one end. The resulting lollipop-shaped shaped structure is called a stem loop and can be recognized by the RNAi machinery and processed intracellularly into short duplex RNAs having siRNA-like properties.

[0053] shRNA can be synthesized in a cell by transcription from a DNA template that has been inserted into an appropriate vector. Useful shRNAs are typically 50-70 nucleotides in length, with two complementary sequences of 19-29 nucleotides separated by a 5-10

nucleotide loop. shRNA construction is generally effected by one of dime methods: annealing of complementary oligonucleotides; promoter-based polymerase chain reaction (PCR); or primer extension. Many vector systems employ RNA Pol III promoters; Pol III-mediated transcription is advantageous because it initiates at a well-defined start-site, produces a non-poly (A) containing transcript and Pol III promoters are active in all cell types. (Brummelkamp *et al.*, (2002) Science 296: 550-553; McIntyre, G. and Fanning, G. (2006) BMC Biotechnology 6: 1-8).

[0054] shRNA-encoding vector systems provide a renewable intracellular source of gene-silencing reagents that can mediate persistent gene silencing after stable integration of the vector into the host genome. Moreover, the shRNA cassette can be readily inserted into retroviral, lentiviral or adenoviral vectors to facility delivery of shRNA into a broad range of cell types, including nondividing primary cultures. Regulatable versions of, shRNA vectors are particularly useful for genetic screens.

[0055] Alternatively, iRNA molecules can be delivered through charge neutralization processes and compositions. For example, US Pat. Publ. Nos.2009/0093425, 2009/0093026, and WO/2014/031575.

[0056] In one embodiment, the disclosure provide methods and compositions for treating a cancer wherein the cancer cells comprise over expression of a BPTF, the method comprising administering a composition comprising a nucleic acid inhibitor of BPTF. Such nucleic acid inhibitors can include, for example, siRNA, shRNA and precursors thereof. For example, the disclosure demonstrates that shRNA can be used to knockdown expression of BPTF and provide beneficial results. Such shRNAs include, but are not limited to, BPTF shRNA1: TGGCTGTGATCGGTGTCAGAATTGGTACC (SEQ ID NO:3; wherein T can be U); BPTF shRNA2: GGTGATGAAGCATAATGCTGTA ATAGAAC (SEQ ID NO:4; wherein T can be U); and BPTF shRNA3 (SEQ ID NO:5; wherein T can be U): ATTTAGATTCATCATAAGGCG as well as any of the forgoing comprising a modified based, charge neutralization moiety and the like. The inhibitory nucleic acids are administered in a therapeutically effective amount.

Date Reçue/Date Received 2021-04-30

[0057] As used herein, the term "therapeutically effective amount" is meant to refer to an amount of a medicament which produces a medicinal effect observed as reduction or reverse in one or more clinical endpoints, growth and/or survival of cancer cell, metastasis of cancer cells in an individual, or reduced resistance of a cancer cells to a chemotherapeutic of first-line anti-cancer agent, when a therapeutically effective amount of the medicament is administered to the individual. Therapeutically effective amounts are typically determined by the effect they have compared to the effect observed when a composition which includes no active ingredient is administered to a similarly situated individual. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. However, the effective amount for a given situation is determined by routine experimentation and is within the judgment of the clinician.

[0058] As used herein, the terms "in combination with" or "in conjunction with" refer to administration of the BPTF modulators of the disclosure with other therapeutic regimens.

[0059] As used herein, the term "susceptible" refers to patients for whom BPTF therapy is an acceptable method of treatment, *e.g.*, patients who are likely to respond positively. Cancer patients susceptible to BPTF therapy express high levels of BPTF relative to those patients not susceptible to BPTF therapy. Cancer patients who are not good candidates for BPTF therapy include cancer patients with tumor samples that lack or have lower levels of BPTF in or on their cancer cells.

[0060] As used herein, the term "detecting" means to establish, discover, or ascertain evidence of an activity (for example, gene expression) or biomolecule (for example, a polypeptide).

[0061] As used herein, the phrase "homologous nucleotide sequence," or "homologous amino acid sequence," or variations thereof, refers to sequences characterized by a homology, at the nucleotide level or amino acid level, of at least a specified percentage and is used interchangeably with "sequence identity". Homologous nucleotide sequences include those sequences coding for

isoforms of proteins. Such isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences include nucleotide sequences encoding for a protein of a species other than humans, including, but not limited to, mammals. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. Homologous amino acid sequences include those amino acid sequences which contain conservative amino acid substitutions and which polypeptides have the same binding and/or activity.

[0062] Percent homology or identity can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, homology between the probe and target is between about 50% to about 60%. In some embodiments, nucleic acids have nucleotides that are about 60%, about 70%, about 80%, about 85%, about 90%, about 92%, about 94%, about 95%, about 97%, about 98%, about 99% and about 100% homologous to SEQ ID NO:1, or a portion thereof. The disclosure further provides partial of full complements of SEQ ID NO:1 or its homologs.

[0063] Homology may also be at the polypeptide level. In some embodiments, polypeptides are about 60%, about 70%, about 80%, about 85%, about 90%, about 92%, about 94%, about 95%, about 97%, about 98%, about 99% and about 100% homologous to SEQ ID NO:2, or a portion thereof.

[0064] As used herein, the term "probe" refers to nucleic acid sequences of variable length. In some embodiments probes comprise at least about 10 and as many as about 6,000 nucleotides. In some embodiments probes comprise at least 12, at least 14, at least 16, at least 18; at least 20, at least 25, at least 50 or at least 75 consecutive nucleotides. Probes are used in the detection of identical; similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from natural or recombinant

sources, are highly specific to the target sequence, and are much slower to hybridize to the target than are oligomers. Probes may be single- or double-stranded and are designed to have specificity in PCR, hybridization membrane-based, in situ hybridization (ISH), fluorescent in situ hybridization (FISH), or ELISA-like technologies.

[0065] As used herein, the term "mixing" refers to the process of combining one or more compounds, cells, molecules, and the like together in the same area. This may be performed, for example, in a test tube, petri dish, or any container that allows the one or more compounds, cells, or molecules, to be mixed.

[0066] As used herein the term "isolated" refers to a polynucleotide, a polypeptide, an antibody, or a host cell that is in an environment different from that in which the polynucleotide, the polypeptide, or the antibody naturally occurs. Methods of isolating cells are well known to those skilled in the art. A polynucleotide, a polypeptide, or an antibody which is isolated is generally substantially purified.

[0067] As used herein, the term "substantially purified" refers to a compound (*e.g.*, either a polynucleotide or a polypeptide or an antibody) that is removed from its natural environment and is at least 60% free, at least 75% free, and at least 90% free from other components with which it is naturally associated.

[0068] As used herein, the term "binding" means the physical or chemical interaction between two or more biomolecules or compounds. Binding includes ionic, non-ionic, hydrogen bonds, Van der Waals, hydrophobic interactions, etc. Binding can be either direct or indirect; indirect being through or due to the effects of another biomolecule or compound. Direct binding refers to interactions that do not take place through or due to the effect of another molecule or compound but instead are without other substantial chemical intermediates.

[0069] As used herein, the term "contacting" means bringing together, either directly or indirectly, one molecule into physical proximity to a second molecule. The molecule can be in any number of buffers, salts, solutions, etc. "Contacting" includes, for example, placing a polynucleotide into a beaker, microtiter plate,

cell culture flask, or a microarray, or the like, which contains a nucleic acid molecule. Contacting also includes, for example, placing an antibody into a beaker, microtiter plate, cell culture flask, or microarray, or the like, which contains a polypeptide. Contacting may take place *in vivo*, ex vivo, or *in vitro*.

[0070] As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a probe, primer, or oligonucleotide will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences will hybridize with specificity to their proper complements at higher temperatures. Generally, stringent conditions are selected to be about 5°C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present in excess, at T_m, 50% of the probes are hybridized to their complements at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C. for short probes, primers or oligonucleotides (*e.g.*, 10 to 50 nucleotides) and at least about 60°C. for longer probes, primers or oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

[0071] As used herein, the term "moderate stringency conditions" refers to conditions under which a probe, primer, or oligonucleotide will hybridize to its target sequence, but to a limited number of other sequences. Moderate conditions are sequence-dependent and will be different in different circumstances. Moderate conditions are well-known to the art skilled and are described in, inter alia, Maniatis *et al.* (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory; 2nd Edition (December 1989)).

[0072] The nucleic acid compositions described herein can be used; for example, to produce polypeptides, as probes for the detection of mRNA in biological samples (*e.g.*, extracts of human cells) or cDNA produced from such samples, to generate additional copies of the polynucleotides, to generate ribozymes or oligonucleotides (single and double stranded), and as single stranded DNA probes or as triple-strand forming oligonucleotides. The probes described herein can be used to, for example, determine the presence or absence of the polynucleotides provided herein in a sample. The polypeptides can be used to generate antibodies specific for a polypeptide associated with cancer, which antibodies are in turn useful in diagnostic methods, prognostic methods, and the like as discussed in more detail herein. Polypeptides are also useful as targets for therapeutic intervention, as discussed in more detail herein. Antibodies of the disclosure may also be used, for example, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies are useful in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). These and other uses are described in more detail below. Antibodies to BPTF are known (*e.g.*, from Bethyl Laboratories, Inc., Montgomery, TX, USA).

[0073] As used herein the term "imaging agent" refers to a composition linked to an antibody, small molecule, or probe of the disclosure that can be detected using techniques known to those of skill in the art. As used herein, the term "evidence of gene expression" refers to any measurable indicia that a gene is expressed.

[0074] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolized

macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers in therapeutic compositions can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles.

[0075] Specific examples of cancers that can be treated by the methods and compositions of the disclosure include, but are not limited to, BPTF associated cancers. As used herein, "BPTF associated cancer" refers to a cancer characterized by cells that differentially express BPTF relative to non-cancerous cells. The disclosure is also applicable to any tumor cell-type where BPTF plays a role in cancer cell growth, tumor formation, cancer cell proliferation, cancer cell metastasis, cell migration, resistance to therapeutics, and angiogenesis. In some embodiments, the cancer is breast cancer, skin cancer, esophageal cancer, liver cancer, pancreatic cancer, prostatic cancer, uterine cancer, cervical cancer, lung cancer, bladder cancer, ovarian cancer, multiple myeloma and melanoma.

[0076] The disclosure provides methods and compositions that provide for the treatment, inhibition, and management of diseases and disorders associated with BPTF overexpression as well as the treatment, inhibition, and management of symptoms of such diseases and disorders. Some embodiments of the invention relate to methods and compositions comprising compositions that treat, inhibit or manage cancer including, without limitation, cancer metastases, cancer cell proliferation, cancer cell growth and cancer cell invasion.

[0077] The disclosure further provides methods including other active ingredients in combination with the BPTF modulators and inhibitors of the disclosure. In some embodiments, the methods further comprise administering one or more conventional cancer therapeutics to the patient. In some embodiments the methods of the disclosure further comprise treating the patient with one or more of chemotherapy, radiation therapy or surgery in combination with a

BPTF modulator or inhibitor. The administration of BPTF in combination with any other therapy can be performed prior to, simultaneously with, or subsequent to the administration of the non-BPTF therapy.

[0078] The disclosure also provides diagnostic and/or imaging methods using the BPTF modulators of the disclosure, particularly BPTF inhibitory antibodies and small inhibitory RNA molecules (*e.g.*, siRNA, shRNA and the like), to diagnose cancer and/or predict cancer progression. In some embodiments, the methods of the disclosure provide methods of imaging and localizing tumors and/or metastases and methods of diagnosis and prognosis of a cancer. In some embodiments, the methods of the disclosure provide methods to evaluate the appropriateness of BPTF-related therapy.

[0079] The disclosure provides BPTF modulators and inhibitors for, *inter alia*, the treatment, diagnosis, detection or imaging of cancer. BPTF modulators or inhibitors are also useful in the preparation of medicaments for the treatment of cancer.

[0080] In some embodiments, the BPTF modulator or inhibitor is an oligonucleotide, a small molecule, a mimetic, or an antibody. In some embodiments, the BPTF modulator inhibits a BPTF biological activity by 25%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, 99% or 100%, as compared to a control. In some embodiments, the BPTF modulator inhibits BPTF expression by at least 25%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 97%; 98%, 99% or 100%, as compared to a control.

[0081] As used herein a BPTF modulator includes any agent that modulates the expression or activity of BPTF. A "modulator" can be antagonistic or agonistic. For treatment of cancers, the BPTF modulator is an inhibitory or antagonistic molecule. Exemplary BPTF inhibitory agents include inhibitory antibodies, small molecule inhibitors (*e.g.*, bromodomain inhibitors), and inhibitor nucleic acid molecules. BPTF inhibitory antibodies include antibodies that inhibit or reduce the biological activity of a BPTF polypeptide (*e.g.*, a polypeptide comprising a sequence of SEQ ID NO:2, a mutant or variant thereof).

[0082] In some embodiments the BPTF modulator is a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a human

antibody, a humanized antibody, a single-chain antibody, or a Fab fragment. The antibody may be labeled with, for example, an enzyme, radioisotope, or fluorophore. In some embodiments, the BPTF modulator is a monoclonal antibody which binds to BPTF consisting of a sequence as set forth in SEQ ID NO:2. In diagnostic applications the antibody can be labeled with a detectable label.

[0083] In some embodiments the antibody is a humanized antibody. Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting the non-human complementary determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as "humanizing"), or, alternatively, (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering"). In the invention, humanized antibodies will include both "humanized" and "veneered" antibodies. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; and in the following scientific publications: Marks *et al.*, Bio/Technology 10; 779-783 (1992); Lonberg *et al.*, Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild *et al.*, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995); Jones *et al.*, Nature 321:522-525 (1986); Morrison *et al.*, Proc. Natl. Acad. Sci; U.S.A., 81:6851-6855 (1984); Morrison and Oi, Adv. Immunol., 44:65-92 (1988); Verhoeyer *et al.*; Science 239:1534-1536 (1988); Padlan, Molec. Immun. 28:489-498 (1991); Padlan, Molec. Immunol. 31(3):169-217 (1994); and Kettleborough, C. A. *et al.*, Protein Eng. 4(7):773-83 (1991).

Date Reçue/Date Received 2021-04-30

[0084] An antibody to BPTF may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the disclosure may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs; radionuclides, or toxins. See, *e.g.*, PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

[0085] In addition to chimeric and humanized antibodies, fully human antibodies can be derived from transgenic mice having human immunoglobulin genes (see, *e.g.*, U.S. Pat. Nos. 6,075,181, 6,091,001, and 6,114,598)

or from phage display libraries of human immunoglobulin genes (see, *e.g.* McCafferty *et al.*, Nature, 348:552-554 (1990). Clackson *et al.*, Nature, 352:624-628 (1991), and Marks *et al.*, J. Mol. Biol., 222:581-597 (1991)).

[0086] Monoclonal antibodies can be prepared using the method of Kohler *et al.* (1975) Nature 256:495-496, or a modification thereof. Typically, a mouse is immunized with a solution containing an antigen. Immunization can be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally. Any method of immunization known in the art may be used to obtain the monoclonal antibodies of the invention. After immunization of the animal, the spleen (and optionally, several large lymph nodes) are removed and dissociated into single cells. The spleen cells may be screened by applying a cell well suspension to a plate or well coated with the antigen of interest. The B cells expressing membrane bound immunoglobulin specific for the antigen bind to the plate and are not rinsed away. Resulting B cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium. The resulting cells are plated by serial or limiting dilution and are assayed for the production of antibodies that specifically bind the antigen of interest (and that

Date Reçue/Date Received 2021-04-30

do not bind to unrelated antigens). The selected monoclonal antibody (mAb)-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

[0087] As an alternative to the use of hybridomas for expression, antibodies can be produced in a cell line such as a CHO or myeloma cell lines, as disclosed in U.S. Pat. Nos. 5,545,403; 5,545,405; and 5,998,144.

Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Immunol. 147:8; Banchereau *et al.* (1991) Clin. Immunol. Spectrum 3:8; and Banchereau *et al.* (1991) Science 251:70.

[0088] The phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural FY region of a native immunoglobulin binding site. See, *e.g.*, Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); Kabat *et al.*, U.S. Dept. of Health and Human Services NaI Publication No. 91-3242 (1991). The phrase "constant region" refers to the portion of the antibody molecule, that confers effector functions. In the present invention, mouse constant regions are substituted by human constant regions. The constant regions of the subject humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region that disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRs of the parent non-human antibody. Such humanized antibodies may be further derivatized to

Date Reçue/Date Received 2021-04-30

facilitate uptake and clearance, *e.g.*, via Ashwell receptors. See, *e.g.*, U.S. Pat. Nos. 5,530,101 and 5,585,089 .

[0089] Humanized antibodies can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Pat. No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions. Antibodies of the present invention can also be produced using human engineering techniques as discussed in U.S. Pat. No. 5,766,886.

[0090] Antibodies of the disclosure may be administered to a subject via *in vivo* therapeutic antibody gene transfer as discussed by Fang *et al.* (2005), Nat. Biotechnol. 23, 584-590. For example recombinant vectors can be generated to deliver a multicistronic expression cassette comprising a peptide that mediates enzyme independent, cotranslational self-cleavage of polypeptides placed between MAb heavy and light chain encoding sequences. Expression leads to stoichiometric amounts of both MAb chains. A preferred example of the peptide that mediates enzyme independent, cotranslational self-cleavage is the foot-and-mouth-disease derived 2A peptide.

[0091] Fragments of the antibodies are suitable for use in the methods of the invention so long as they retain the desired

Date Reçue/Date Received 2021-04-30

affinity of the full-length antibody. Thus, a fragment of an anti-BPTF antibody will retain the ability to bind to BPTF. Such fragments are characterized by properties similar to the corresponding full-length anti-BPTF antibody, that is, the fragments will specifically bind a human BPTF antigen expressed on the surface of a human cell.

[0092] In some embodiments, the antibodies bind to one or more epitopes in a domain of BPTF. In some embodiments, the antibodies modulate one or more BPTF related biological activities. In some embodiments the antibodies inhibit one or more of cancer cell growth, tumor formation, and cancer cell proliferation.

[0093] Antibodies are defined to be "specifically binding" if: 1) they exhibit a threshold level of binding activity, and/or 2) they do not significantly cross-react with known related polypeptide molecules. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard, analysis (Scatchard, Ann. NY Acad. Sci. 51: 660-672, 1949).

[0094] The disclosure provides methods for treating and/or preventing cancer or symptoms of cancer in a subject comprising administering to the subject a therapeutically effective amount of one or more BPTF inhibitors of the disclosure. In some embodiments the cancer is a cancer associated with BPTF overexpression. In some embodiments, the cancer is breast cancer, skin cancer, esophageal cancer, liver cancer, pancreatic cancer, prostatic cancer, uterine cancer, cervical cancer, lung cancer, bladder cancer, ovarian cancer, multiple myeloma or melanoma. In some embodiments, the cancer is in a non-hormonally regulated tissue.

[0095] A therapeutically effective amount of the inhibitor compound can be determined empirically, according to procedures well known to medicinal chemists, and will depend, inter alia, on the age of the patient, severity of the condition, and on the ultimate pharmaceutical formulation desired. Administration of the modulators of the invention can be carried out, for example, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue, orally, topically, intranasally, intraperitoneally, parenterally,

intravenously, intralymphatically, intratumorly, intramuscularly, interstitially, intra-arterially, subcutaneously, intraoccularly, intrasynovial, transepithelial, and transdermally. In some embodiments, the inhibitors are administered by lavage, orally or inter-arterially. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow or sustained release polymeric devices. As discussed above, the therapeutic compositions of this disclosure can also be administered as part of a combinatorial therapy with other known anti-cancer agents or other known anti-bone disease treatment regimen.

[0096] The disclosure further provides methods of modulating a BPTF-related biological activity in a patient. The methods comprise administering to the patient an amount of a BPTF inhibitor effective to inhibit one or more BPTF biological activities. Suitable assays for measuring BPTF biological activities are set forth *supra* and *infra*.

[0097] The disclosure also provides methods of inhibiting cancer cell growth in a patient in need thereof comprising administering a therapeutically effective amount of one or more BPTF inhibitors to the patient. Suitable assays for measuring BPTF-related cell growth are known to those skilled in the art and are set forth *supra* and *infra*.

[0098] The disclosure further provides methods of inhibiting cancer in a patient in need thereof. The methods comprise determining if the patient is a candidate for BPTF therapy as described herein (*e.g.*, wherein BPTF is overexpressed) and administering a therapeutically effective amount of one or more BPTF inhibitors to the patient if the patient is a candidate for BPTF therapy. If the patient is not a candidate for BPTF therapy, the patient is treated with conventional cancer treatment.

[0099] The disclosure further provides methods of inhibiting cancer in a patient, diagnosed or suspected of having a cancer. The methods comprise administering a therapeutically effective amount of one or more BPTF inhibitors to the patient.

[00100] The disclosure also provides methods of modulating one or more symptoms of cancer in a patient comprising administering to

said patient a therapeutically effective amount of the BPTF inhibitory compositions described herein.

[00101] The disclosure also provides methods for inhibiting migration of cancer cells in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a BPTF inhibitor. Suitable assays for measuring BPTF-related cell migration are known to those skilled in the art.

[00102] The disclosure also provides methods for prophylactically treating a patient who is predisposed to develop cancer, a cancer metastasis or who has had a metastasis and is therefore susceptible to a relapse or recurrence. The methods are particularly useful in high-risk individuals who, for example, have a family history of cancer or of metastasizing tumors, or show a genetic predisposition for a cancer metastasis. In some embodiments the tumors are BPTF-related tumors. A "BPTF-related" tumor or caner is a tumor or cancer which shows an increased expression of BPTF compared to the same non-tumor or cancer cell type. Additionally, the methods are useful to prevent patients from having recurrences of BPTF-related tumors who have had BPTF-related tumors removed by surgical resection or treated with a conventional cancer treatment.

[00103] The disclosure also provides methods of inhibiting cancer progression and/or causing cancer regression comprising administering to the patient a therapeutically effective amount of a BPTF inhibitor.

[00104] In some embodiments, the patient in need of anti-cancer treatment is treated with the BPTF inhibitor of the disclosure in conjunction with chemotherapy and/or radiation therapy. For example, following administration of the BPTF inhibitor, the patient may also be treated with a therapeutically effective amount of anti-cancer radiation. In some embodiments chemotherapeutic treatment is provided in combination with BPTF inhibitor. In some embodiments BPTF inhibitors are administered in combination with chemotherapy and radiation therapy.

[00105] Methods of treatment comprise administering single or multiple doses of one or more BPTF inhibitors to the patient. In some embodiments the BPTF inhibitors are administered as injectable pharmaceutical compositions that are sterile, pyrogen free and

comprise the BPTF modulators in combination with a pharmaceutically acceptable carrier or diluent.

[00106] In some embodiments, the therapeutic regimens of the disclosure are used with conventional treatment regimens for cancer including, without limitation, surgery, radiation therapy, hormone ablation and/or chemotherapy. Administration of the BPTF inhibitors of the disclosure may take place prior to, simultaneously with, or after conventional cancer treatment. In some embodiments, two or more different BPTF inhibitors are administered to the patient.

[00107] In some embodiments the amount of BPTF inhibitor administered to the patient is effective to inhibit one or more of cancer cell growth, tumor formation, cancer cell proliferation, cancer cell metastasis, cancer cell migration, angiogenesis, and the like. In some embodiments, the amount of BPTF inhibitor administered to the patient is effective to increase cancer cell death through apoptosis.

[00108] In some embodiments the disclosure provides compositions comprising two or more BPTF inhibitors to provide still improved efficacy against cancer. In, some embodiments the BPTF inhibitors are inhibitory nucleic acid molecules (*e.g.*, shRNA, siRNA and antisense molecules) or inhibitory antibodies. Concurrent administration of two or more therapeutic agents does not require that the agents be administered at the same time or by the same route, so long as there is an overlap in the time period during which the agents are exerting their therapeutic effect. Simultaneous or sequential administration is contemplated, as is administration on different days or weeks.

[00109] In some embodiments the methods of the disclosure contemplate the administration of combinations, or "cocktails", of different BPTF inhibitory agents.

[00110] Cancer chemotherapeutic agents that can be used in combination with BPTF inhibitory agents of the disclosure include, without limitation, alkylating agents, such as carboplatin and cisplatin; nitrogen mustard alkylating agents; nitrosourea alkylating agents, such as carmustine (BCNU); antimetabolites, such as methotrexate; folinic acid; purine analog antimetabolites, mercaptopurine; pyrimidine analog antimetabolites, such as

fluorouracil (5-FU) and gemcitabine (Gemzar®); hormonal antineoplastics, such as goserelin, leuprolide, and tamoxifen; natural antineoplastics, such as aldesleukin, interleukin-2, docetaxel, etoposide (VP-16), interferon alfa, paclitaxel (Taxol®), and tretinoin (ATRA); antibiotic natural antineoplastics, such as bleomycin, dactinomycin, daunorubicin, doxorubicin, daunomycin and mitomycins including mitomycin C; and vinca alkaloid natural antineoplastics, such as vinblastine, vincristine, vindesine; hydroxyurea; aceglatone, adriamycin, ifosfamide, enocitabine, epitiostanol, aclarubicin, ancitabine, procarbazine hydrochloride, carboquone, carboplatin, carmofur, chromomycin A3, antitumor polysaccharides, antitumor platelet factors, cyclophosphamide (Cytoxin®), Schizophyllan, cytarabine (cytosine arabinoside), dacarbazine, thioinosine, thiotepa, tegafur, dolastatins, dolastatin analogs such as auristatin, CPT-11 (irinotecan), mitozantrone, vinorelbine, teniposide, aminopterin, caminomycin, esperamicins (See, *e.g.*, U.S. Pat. No. 4,675,187), neocarzinostatin, OK-432, bleomycin, furtulon, broxuridine, busulfan, honvan, peplomycin, bestatin (Ubenimex®), interferon-β, mepitiostane, mitobronitol, melphalan, laminin peptides, lentinan, Coriolus versicolor extract, tegafur/uracil, estramustine (estrogen/mechlorethamine).

[00111] Additional agents which may be used as therapy for cancer patients include EPO, G-CSF, ganciclovir; antibiotics, leuprolide; meperidine; zidovudine (AZT); interleukins 1 through 18, including mutants and analogues; interferons or cytokines, such as interferons alpha, beta, and gamma hormones, such as luteinizing hormone releasing hormone (LHRH) and analogues and, gonadotropin releasing hormone (GnRH); growth factors, such as transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), nerve growth factor (NGF), growth hormone releasing factor (GHRF), epidermal growth factor (EGF), fibroblast growth factor homologous factor (FGFHF), hepatocyte growth factor (HGF), and insulin growth factor (IGF); tumor necrosis factor-α & β (TNF-α& β); invasion inhibiting factor-2 (IIF-2); bone morphogenetic proteins 1-7 (BMP 1-7); somatostatin; thymosin-alpha-1; gamma-globulin; superoxide dismutase (SOD); complement factors; anti-angiogenesis factors;

antigenic materials; and pro-drugs. In addition, a BPTF inhibitor can be combined with a targeted agent, including but not limited to those targeting BRAF (vemurafenib or dabrafenib), MEK (trametinib), HER2 (*e.g.*, herceptin), and EGFR (*e.g.*, gefitinib). Other targeting combinations include a BPTF inhibitor and one or more of the following: Erbitux (cetuximab), Yervoy (ipilimumab) and pertuzumab. Examples of such therapies also include, by no way of limitation, small-molecule kinase inhibitors such as Imatinib (Gleevec), Sunitinib (Sutent), Sorafenib (Nexavar), Erlotinib (Tarceva), , Dasatinib (Sprycel), Nilotinib (Tasigna), Lapatinib (Tykerb), Crizotinib (Xalkori), Ruxolitinib (Jakafi), , Vandetanib (Caprelsa), Pazopanib (Votrient), afatinib, alisertib, amuvatinib, axitinib, bosutinib, brivanib, canertinib, cabozantinib, cediranib, crenolanib, dacomitinib, danusertib, dovitinib, foretinib, ganetespib, ibrutinib, iniparib, lenvatinib, linifanib, linsitinib, masitinib, momelotinib, motesanib, neratinib, niraparib, oprozomib, olaparib, pictilisib, ponatinib, quizartinib, regorafenib, rigosertib, rucaparib, saracatinib, saridegib, tandutinib, tasocitinib, telatinib, tivantinib, tivozanib, tofacitinib, vatalanib, veliparib, vismodegib, volasertib, BMS-540215, BMS777607, JNJ38877605, TKI258, GDC-0941 (Folkes, *et al.*, J. Med. Chem. 2008, 51, 5522), BZE235, and others.

[00112] A prodrug refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic or non-cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into an active or the more active parent form. See, *e.g.*, Wilman, "Prodrugs in Cancer Chemotherapy" Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). Prodrugs include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, b-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing

prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use herein include, but are not limited to, those chemotherapeutic agents described above.

[00113] In some embodiments, the methods and compositions of the disclosure are particularly useful in breast cancer, brain cancer (glioblastoma multiforme), skin cancer, esophageal cancer, liver cancer, pancreatic cancer, prostatic cancer, uterine cancer, cervical cancer, lung cancer, bladder cancer, ovarian cancer, multiple myeloma and melanoma.

[00114] The disclosure also provides pharmaceutical compositions comprising one or more of the BPTF inhibitors described herein and a pharmaceutically acceptable carrier. In some embodiments the pharmaceutical compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier. Pharmaceutically acceptable salts can also be present in the pharmaceutical composition, *e.g.*, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington: The Science and Practice of Pharmacy (1995) Alfonso Gennaro, Lippincott, Williams, & Wilkins.

[00115] The disclosure also provides methods for detecting BPTF. In some embodiments, BPTF can be detected in a patient *in vivo* or in a patient sample *in vitro*. In some embodiments the method comprises administering to a patient a composition comprising one or more BPTF inhibitors that bind to a BPTF polypeptide or nucleic acid and detecting the localization of the BPTF agent that is labeled with a detectable label in the patient. In some embodiments the patient sample comprises cancer cells. In some embodiments the BPTF inhibitor is linked to an imaging agent or is detectably labeled. In some embodiments, the BPTF inhibitor or binding agent is a BPTF antibody conjugated to an imaging agent and is

administered to a patient to detect one or more tumors or to determine susceptibility of the patient to BPTF therapy. The labeled antibodies will bind to BPTF polypeptides in or on cells and thereby accumulate at a tumor site. Using standard imaging techniques, the site of the tumors can be detected.

[00116] The disclosure also provides methods of imaging/detecting cells or tumors expressing or overexpressing BPTF comprising contacting a composition comprising an agent (*e.g.*, a nucleic acid or antibody) that binds to BPTF with a sample and detecting the presence of the BPTF in the sample. In some embodiments the sample is a patient sample. In some embodiments the patient sample comprises cancer cells.

[00117] Methods of detection are well known to those of skill in the art. For example, methods of detecting polynucleotides include, but are not limited to PCR, Northern blotting, Southern blotting, RNA protection, and DNA hybridization (including in situ hybridization). Methods of detecting polypeptides include, but are not limited to, Western blotting, ELISA, enzyme activity assays, slot blotting, peptide mass fingerprinting, electrophoresis, immunochemistry and immunohistochemistry. Other examples, of detection methods include, but are not limited to, radioimmunoassay (RIA), chemiluminescence immunoassay, fluoroimmunoassay, time-resolved fluoroimmunoassay (TR-FIA), two color fluorescent microscopy, or immunochromatographic assay (ICA), all well known by those of skill in the art. In some embodiments, polynucleotide expression is detected using PCR methodologies and polypeptide production is detected using ELISA technology.

[00118] Suitable probes for Northern blot hybridization of a given nucleic acid can be produced from the nucleic acid sequences of BPTF (*e.g.*, SEQ ID NO:1). Methods for preparation of labeled DNA and RNA probes, and the conditions for hybridization thereof to target nucleotide sequences, are described in Molecular Cloning: A Laboratory Manual, J. Sambrook *et al.*, eds., 2nd edition, Cold Spring Harbor Laboratory Press, 1989, Chapters 10 and 11.

[00119] In one example, the nucleic acid probe can be labeled with, *e.g.*, a radionucleotide, such as ^{3}H, ^{32}P, ^{33}P, ^{14}C, or ^{35}S; a heavy metal; or a ligand capable of functioning as a specific

binding pair member for a labeled ligand (*e.g.*, biotin, avidin or an antibody), a fluorescent molecule, a chemiluminescent molecule, or an enzyme. Probes can be labeled to high specific activity by nick translation, random priming, or other methods known to one of skill in the art. For example, by replacing preexisting nucleotides with highly radioactive nucleotides according to the nick translation method, it is known to prepare ^{32}P-labeled nucleic acid probes with a specific activity well in excess of 10^8 cpm/microgram.

[00120] Autoradiographic detection of hybridization can then be performed by exposing hybridized filters to photographic film. Densitometric scanning of the photographic films exposed by the hybridized filters provides an accurate measurement of miRNA transcript levels. In another embodiment, gene transcript levels can be quantified by computerized imaging systems, such the Molecular Dynamics 400-B 2D Phosphorimager available from Amersham Biosciences, Piscataway, N.J.

[00121] In another embodiment, the random-primer method can be used to incorporate an analogue, for example, the dTTP analogue 5-(*N*-(*N*-biotinyl-epsilon-aminocaproyl)-3-aminoallyl)deoxyuridine triphosphate, into the probe molecule. The biotinylated probe oligonucleotide can be detected by reaction with biotin-binding proteins, such as avidin, streptavidin, and antibodies (*e.g.*, anti-biotin antibodies) coupled to fluorescent dyes or enzymes that produce color reactions.

[00122] In a further embodiment, determining the levels of an expression can be accomplished using the technique of *in situ* hybridization. This technique requires fewer cells than the Northern blotting technique, and involves depositing whole cells onto a microscope cover slip and probing the nucleic acid content of the cell with a solution containing radioactive or otherwise labeled nucleic acid (*e.g.*, cDNA or RNA) probes. This technique is particularly well-suited for analyzing tissue biopsy samples from subjects. The practice of the in situ hybridization technique is described in more detail in U.S. Pat. No. 5,427,916,

Date Reçue/Date Received 2021-04-30

[00123] The relative number of gene transcripts in cells can also be determined by reverse transcription of gene transcripts, followed by amplification of the reverse-transcribed transcripts by polymerase chain reaction (RT-PCR). The levels of gene transcripts can be quantified in comparison with an internal standard, for example, the level of mRNA from a "housekeeping" gene present in the same sample. A suitable "housekeeping" gene for use as an internal standard includes, *e.g.*, myosin or glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The methods for quantitative RT-PCR and variations thereof are within the skill in the art. In another embodiment, a high throughput stem loop real-time quantitative polymerase chain reaction (RT-qPCR) is used to detect miRNA expression. See Mestdagh *et al.*, *Nucleic Acid Research* 36(21) (2008)).

[00124] An "expression profile" or "hybridization profile" of a particular sample is essentially a fingerprint of the state of the sample; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. That is, normal tissue may be distinguished from a cancer tissue, and within a cancer tissue, different prognosis states (good or poor long term survival prospects, for example) may be determined. By comparing expression profiles of a cancer tissue in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. The identification of sequences that are differentially expressed in a cancer tissue or normal tissue, as well as differential expression resulting in different prognostic outcomes, allows the use of this information in a number of ways. For example, a particular treatment regime may be evaluated (*e.g.*, to determine whether a chemotherapeutic drug acts to improve the long-term prognosis in a particular patient). Similarly, diagnosis may be done or confirmed by comparing patient samples with the known expression profiles. Furthermore, these gene expression profiles (or individual genes) allow screening of drug candidates that suppress the cancer

expression profile or convert a poor prognosis profile to a better prognosis profile.

[00125] According to the expression profiling methods described herein, total RNA from a sample from a subject suspected of having a cancer (*e.g.*, melanoma, breast cancer, GBM) is quantitatively reverse transcribed to provide a set of labeled target oligodeoxynucleotides complementary to the RNA in the sample. The target oligodeoxynucleotides are then hybridized to a microarray comprising RNA-specific probe oligonucleotides to provide a hybridization profile for the sample. The result is a hybridization profile for the sample representing the expression pattern of genes in the sample. The hybridization profile comprises the signal from the binding of the target oligodeoxynucleotides from the sample to the BPTF-specific probe oligonucleotides in the microarray. The profile may be recorded as the presence or absence of binding (signal vs. zero signal). Typically the profile recorded includes the intensity of the signal from each hybridization. The profile is compared to the hybridization profile generated from a control sample. An alteration in the signal is indicative of a chemotherapy response in the subject.

[00126] Other techniques for measuring gene expression are also within the skill in the art, and include various techniques for measuring rates of RNA transcription and degradation, including Rnase Protection Assays, Nuclear run-ons, slot blotting, etc.

[00127] In another embodiment, the disclosure provides a method for prognosticating the presence of a cancer in a subject. The method comprises the step of determining whether or not BPTF is over-expressed or under-expressed in a biological sample from the subject, relative to the expression of BPTF from one or more control samples. In a particular embodiment, the level of over-expression of BPTF from a subject's biological sample in comparison to a control biological sample indicates whether the subject's sample is malignant. The cancer can be, for example, GBM, melanoma or breast cancer.

[00128] In yet another embodiment, the disclosure provides a method of determining the progression of cancer in a subject. The method comprises the step of measuring the expression level of BPTF

from biological samples taken from patient having a cancer at various time points, such that the change in the expression level of BPTF between samples from the different time points indicates the progression or recovery from the cancer in the subject. In a particular embodiment, if the level of expression of BPTF is increasing between earlier and later time points this indicates that the subject's cancer is progressing to later stages of cancer. In an alternate embodiment, if the level of expression of BPTF is decreasing between earlier and later time points this indicates that the subject's cancer is in the process of remission. In a further embodiment, said cancer is melanoma, breast cancer or GBM.

[00129] In yet another embodiment, the disclosure provides a method of determining the survival rate of a subject with cancer. The method comprises the step of measuring the level of BPTF from biological samples taken from patient having a cancer at various time points, such that the change in the level of BPTF between samples from the different time points indicates a decreased or increased survival rate of the subject. In a particular embodiment, if the level of expression or over-expression of BPTF is increasing between earlier and later time points this would indicate that the subject's survival rate is decreasing. In an alternate embodiment, if the level of BPTF is decreasing between earlier and later time points this would indicate that the subject's survival rate is improving. In a further embodiment, said cancer is melanoma, breast cancer or GBM. The level of BPTF can be determined through protein detection, DNA detection techniques as well as measuring RNA expression.

[00130] In another embodiment, the disclosure provides a method of treating a cancer, the method comprising administering an effective amount of an agent that inhibits the expression or activity of BPTF.

[00131] The terms "treat", "treating" and "treatment", as used herein, refer to ameliorating symptoms associated with a disease or condition, for example, a melanoma, including preventing or delaying the onset of the disease symptoms, and/or lessening the severity or frequency of symptoms of the disease or condition. The terms "subject" and "individual" are defined herein to include

animals, such as mammals, including but not limited to, primates, cows, sheep, goats, horses, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent, or murine species. In a preferred embodiment, the mammal is a human.

[00132] As used herein, an "effective amount" of BPTF inhibitor is an amount sufficient to inhibit proliferation or invasiveness of a cancer cell in a subject suffering from a cancer. One skilled in the art can readily determine an effective amount of a BPTF inhibitor to be administered to a given subject, by taking into account factors, such as the size and weight of the subject; the extent of disease penetration; the age, health and sex of the subject; the route of administration; and whether the administration is regional or systemic.

[00133] Cancers that may be treated by compositions comprising polynucleotides comprising inhibitory BPTF agents and/or agents that decrease BPTF expression, include, tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may be comprised of non-solid tumors (such as leukemias and lymphomas) or may be solid tumors.

[00134] In a particular embodiment, the disclosure provides a method of treating cancer, such as melanoma, breast cancer or GBM, in a subject, the method comprising administering an effective amount of an agent that inhibits the expression or activity of BPTF. In another embodiment, the agent is a shRNA. In another embodiment, the agent is a double-stranded miRNA mimic. miRNA mimic technology is well known in the art. See *e.g.*, Wang, Z., 2009, miRNA mimic technology, In MicroRNA Interference Technologies, pages 93-100, Springer-Link Publications. In another embodiment, the agent is an oligonucleotide based BPTF drug.

[00135] Expression vectors encoding shRNA or miRNA molecules to BPTF can be delivered to cells of a subject for the treatment or prevention of a cancer. The nucleic acid molecules are delivered to the cells of a subject in a form in which they can be taken up and are advantageously expressed so that therapeutically effective levels can be achieved. Expression vectors that are able to express BPTF shRNA are commercially available from various vendors.

[00136] Methods for delivering polynucleotides comprising inhibitory nucleic acid agents that decrease BPTF expression to the cell include using a delivery system, such as liposomes, polymers, microspheres, gene therapy vectors, modified nucleic acids (*e.g.*, charge neutralized nucleic acids) and naked DNA vectors.

[00137] Transducing viral (*e.g.*, retroviral, adenoviral, lentiviral and adeno-associated viral) vectors can be used for somatic cell gene therapy, especially because of their high efficiency of infection and stable integration and expression (see, *e.g.*, Cayouette *et al.*, *Human Gene Therapy* 8:423-430 (1997); Kido *et al.*, *Current Eye Research* 15:833-844 (1996); Bloomer *et al.*, *Journal of Virology* 71:6641-6649 (1997); Naldini *et al.*, *Science* 272:263-267 (1996); and Miyoshi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:10319 (1997)). For example, a polynucleotide encoding an inhibitory nucleic acid to BPTF can be cloned into a retroviral vector and its expression can be driven from an endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Other viral vectors that can be used include, for example, a vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, *Human Gene Therapy* 15-14, (1990); Friedman, *Science* 244:1275-1281 (1989); Eglitis *et al.*, *BioTechniques* 6:608-614 (1988); Tolstoshev *et al.*, *Current Opinion in Biotechnology* 1:55-61 (1990); Sharp, *The Lancet* 337:1277-1278 (1991); Cornetta *et al.*, *Nucleic Acid Research and Molecular Biology* 36(31):1-322 (1987); Anderson, *Science* 226:401-409 (1984); Moen, *Blood Cells* 17:407-416 (1991); Miller *et al.*, *Biotechnology* 7:980-990 (1989); Le Gal La Salle *et al.*, *Science* 259:988-990 (1993); and Johnson, *Chest* 107:77S-83S (1995)). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al.*, *N. Engl. J. Med* 323:370 (1990); Anderson *et al.*, U.S. Pat. No.5,399,346; Gruber *et al.*, U.S. Pat. Publ. No. 2011/0287020A1). Non-viral approaches can also be employed for the introduction of an inhibitory nucleic acid to BPTF based therapeutic to a cell of a patient diagnosed as having a neoplasia. For example, a polynucleotide comprising an inhibitory nucleic acid to BPTF can be introduced into a cell by

administering the nucleic acid in the presence of cationic lipid (Feigner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 84:7413 (1987); Ono *et al.*, *Neuroscience Letters* 17:259 (1990); Brigham *et al.*, *Am. J. Med. Sci.* 298:278 (1989); and Staubinger *et al.*, *Methods in Enzymology* 101:512 (1983)); asialoorosoinucoid-polylysine conjugation (Wu *et al. Journal of Biological Chemistry* 263:14621 (1988); Wu *et al.*, *Journal of Biological Chemistry* 264:16985 (1989); or by micro-injection under surgical conditions (Wolff *et al.*, *Science* 247:1465 (1990). A polynucleotide comprising an inhibitory nucleic acid to BPTF and/or an agent that inhibits BPTF expression can be administered in combination with a liposome and protamine.

[00138] In another embodiment, the disclosure provides therapeutic compositions comprising polynucleotides comprising an inhibitory nucleic acid to BPTF that inhibit the expression of BPTF for the treatment of a cancer, such as melanoma. In another embodiment, the disclosure provides a pharmaceutical composition comprising an agent that inhibits the expression of BPTF.

[00139] Polynucleotides comprising an inhibitory nucleic acid to BPTF and/or agents that inhibit the expression of BPTF may be administered as part of a pharmaceutical composition. The pharmaceutical composition is preferably sterile and contains a therapeutically effective amount of a polynucleotide molecule comprising an inhibitory BPTF nucleic acid and/or an agent that inhibits the expression of BPTF in a unit of weight or volume suitable for administration to a subject.

[00140] The therapeutic polynucleotide molecule comprising an inhibitory BPTF nucleic acid and/or agents that inhibit the expression of BPTF may be administered with a pharmaceutically-acceptable carrier, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a cancer.

[00141] Carrier as used herein includes pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable

carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[00142] Polynucleotides comprising an inhibitory BPTF nucleic acid and/or agents that inhibits the expression of BPTF may also be entrapped in microcapsules prepared, for example, by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers comprising a polynucleotide comprising an inhibitory BPTF nucleic acid and/or an agent which inhibits BPTF expression, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over

100 days, certain hydrogels release of molecules for shorter time periods.

[00143] In another embodiment, the pharmaceutical compositions comprising polynucleotides comprising an inhibitory BPTF nucleic acid and/or agents that inhibits the expression of BPTF are administered in conjunction with other therapeutic agents. "Conjunction" with respect to administering other therapeutic agents, refers to agents that may administered prior to, concurrently, or subsequent to pharmaceutical compositions comprising polynucleotides comprising an inhibitory BPTF nucleic acid and/or agents which inhibit BPTF expression.

[00144] In another embodiment, the disclosure provides a kit for determining a subject likelihood of having cancer and/or progression of cancer, said kit comprising: a) an oligonucleotide complementary to BPTF; and b) optionally, reagents for the formation of the hybridization between said oligonucleotide and BPTF. In another embodiment, the kit optionally includes directions for monitoring the nucleic acid molecule levels of a marker in a biological sample derived from a subject. In another embodiment, the kit comprises a sterile container which contains the primer, probe, or other detection regents; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container form known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding nucleic acids. The instructions will generally include information about the use of the primers or probes described herein and their use in diagnosing a cancer. Preferably, the kit further comprises any one or more of the reagents described in the diagnostic assays described herein. In other embodiments, the instructions include at least one of the following: description of the primer or probe; methods for using the enclosed materials for the diagnosis of a cancer; precautions; warnings; indications; clinical or research studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

[00145] In another embodiment, the disclosure provides an apparatus for determining the expression levels of BPTF, said apparatus comprising a solid support, wherein a surface of said solid support is linked to an oligonucleotide complementary to BPTF. In one embodiment, the apparatus is a micro-array. The examples of solid support include, but are not limited to, a glass or nitro-cellulose slide that is used to bind nucleic acids.

[00146] The following examples are intended to illustrate but not limit the disclosure. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLES

[00147] **Cell culture and transfection.** C8161.9 and 1205-Lu human melanoma and B16-F10 murine melanoma cells were obtained as described (Bagheri *et al.*, 2006; Dar *et al.*, 2011). U251 and LN18 glioblastoma cell lines were purchased from ATCC (Manassas, VA). C8161.9 cells were grown in DMEM/F12 with 5% fetal bovine serum (FBS) (Invitrogen Life Technologies, Carlsbad, CA); 1205-Lu cells were grown in TU2% medium; and B16-F10 cells were grown in RPMI-1640 with 5% FBS. U251 and LN18 were grown in DMEM with 5% FBS. All cells were grown at 37°C in an atmosphere containing 5% CO_2. Transient transfection was carried out by Lipofectamine-2000 (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol.

[00148] **Plasmids.** Plasmids pcMV6-BPTF, pcMV6-Entry and BPTF targeting shRNA vector set (4 clones) pGFP-V-RS were purchased from Origene (Origene Technologies Rockville MD). BPTF shRNA 1 and BPTF shRNA 2 were used from this set for the murine cell line. The lentiviral-based shRNA vector set (5 clones) targeting BPTF (RHS4533_NM_0059) was purchased from Openbiosystems (Lafayette, CO).

[00149] **RNA extraction and cDNA synthesis**. RNA extraction and cDNA synthesis were performed using techniques common in the art.

[00150] **Quantitative real-time PCR (qRT-PCR).** mRNAs were assayed using the TaqMan Gene Expression Assays in accordance with the manufacturer's instructions (Applied Biosystems). TaqMan probes for BPTF, HPRT1, CCND2, BCL-XL, TWIST1, RAB14, CEBPB, CHI3L1, DLL3,

Date Reçue/Date Received 2021-04-30

OLIG2, PDGFRA and BCL2 were purchased from Applied Biosystems (Foster City, CA).

[00151] Cell viability, colony formation, cell cycle analyses, and BRAF inhibitor treatment. Cell viability and colony formation were performed and cell cycle analysis was performed as described using common techniques in the art. Cells were treated with varying concentrations of vemurafenib for 72 hrs or dabrafenib (Chemitek, Indianapolis, IN) for 48 hrs or as indicated. DMSO was used as a vehicle.

[00152] Western blot analysis. Western blot analysis was performed. Target proteins were detected by using specific antibodies against BPTF (Bethyl Laboratories Montgomery, TX), BCL2, and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA), and ERK1/2, pERK1/2, CCND2, BCL-XL, p90RSK (Cell Signaling Technology, Danvers, MA).

[00153] Lentiviral transduction and stable cell generation. Selected shRNAs cloned into the pLKO1-vector were co-transfected into 293T cells along with expression vectors containing the GAG/POL, REV and VSVG genes. Lentiviruses were harvested 48 hrs after transfection. Sub-confluent human melanoma or glioblastoma cells were infected with each harvested lentiviruses in the presence of 8 µg/ml of polybrene, and were selected in 1µg/ml of puromycin at 48 hrs post-infection in their respective culture medium. B16-F10 cells were transfected with BPTF shRNA1, BPTF shRNA 2 or Neg shRNA vectors and stable transformants were selected with 2 ug/ml of puromycin.

[00154] Invasion assays. A Matrigel assay for tumor invasion was performed using routine techniques. For B16-F10, 1205-Lu and C8161.9 cells, insert chambers were coated with 15µl matrigel at 6mg/ml protein, 17µl and 7mg/ml for C8161.9 cells and 15µl and 5mg/ml for 1205-Lu and U251 cell lines.

[00155] Quantification of DNase I sensitivity. Cells were harvested and resuspended in ice-cold lysis buffer (100mM KCL, 50mM Tris-CL [pH 7.9], 50% [vol/vol] glycerol, 200mM β-mercaptoethanol, and 5mM $MgCl_2$) and incubated for 10 minutes. Nuclei were recovered from the lysed cells by subjecting the suspension to centrifugation at 13,000*g* for 15 minutes at 4°C and resuspending the cells in

Buffer A (50mM Tris-Cl [pH 7.9], 3mM $MgCl_2$, 0.2mM phenylmethylsulfonyl fluoride, 100mM NaCl, and 1mM dithiothreitol). Nuclei were digested with DNase I for 3 minutes at room temperature in Buffer A. The samples were then treated with proteinase K and the DNA was recovered using the QIAGEN PCR purification kit (Qiagen, Valencia, CA). DNase-treated DNA was subjected to qPCR using specific primers for BCL2 and BCL-XL.

[00156] Tissue arrays and immunostaining. The tissue microarrays utilized were previously created using core diameters of 1.0 mm taken from the paraffin blocks. Slides were prepared from formalin-fixed tissue microarrays and stained with anti-human BPTF antibody at a 1:100 dilution (Bethyl Laboratories Montgomery, TX). Microwave antigen retrieval was conducted in 10 mM citrate buffer, pH 6.0. Endogenous peroxidase was blocked with 3% H_2O_2, and additional blocking was performed with normal rabbit serum. The primary antibody was diluted in 1.0% BSA in PBS and applied overnight at 4°C. Antibody staining was observed by using biotin-labeled anti-goat IgG and avidin-biotin (Vector Laboratories, Burlingame, CA) followed by diaminobenzidine. Sections were counterstained with hematoxylin.

[00157] Digital evaluation of immunohistochemical staining. The whole slide image of tissue microarray sections was captured using a Mirax MIDI high-resolution scanning system (Carl Zeiss Micro Imaging, Jena, Germany). The digitization process was controlled via software using a Marlin CCD Camera (Allied Vision Technologies GmbH, Germany) with a Zeiss Plan-Apopchromat 20x/.8NA objective (Carl Zeiss Optronics GmBh, Oberkochen, Germany) to generate images at a resolution of .32 microns/pixel. Regions with an identifiable melanocytic lesion were selected for evaluation, and immunohistochemical staining was calculated by applying a segmentation feature with two different phase measurement masks recognizing nuclei (hematoxylin-stained) and cytoplasm (brown-immunostained). Image analysis was performed by computer-assisted color segmentation to determine the percentage of positive color-expressing pixels. For each positive pixel, the intensity (defined as the average of red, green and blue color values) was calculated for subsequent analysis. Statistical methods used to

assess the significance of various prognostic factors on melanoma outcome were previously described (Bagheri *et al.*, 2006).

[00158] Gene expression profiling. Total RNA was extracted from cells using the RNeasy kit (Qiagen, Valencia, CA). Ten micrograms of total RNA, along with universal mouse reference RNA (Stratagene, Santa Clara, CA), was converted to aminoallyl-modified cDNA by oligo(dT)-primed polymerization using SuperScript II reverse transcriptase (Invitrogen), coupled to *N*-hydroxysuccinimidyl esters of Cy3 or Cy5 (Amersham Pharmacia, Pittsburgh, PA), and then hybridized to a microarray slide as described (Haqq *et al.*, 2005). After linear normalization, log (base 2) transformation, and supervised hierarchical clustering, the resulting cluster data table was imported into the significance analysis of microarrays software package. Delta was chosen to limit the output gene list so that 5% predicted false positives would be included.

[00159] FISH and microscopy. BAC clones RP11-304I14, RP11-1134M2, RP11-29C18 and CTD-2314M10 were used to detect the BPTF locus on 17q24.3 (February 2009 freeze of the UCSC Genome Browser, http://genome.ucsc.edu). All clones were obtained from the Children's Hospital of Oakland Research Institute (CHORI). BAC DNA was prepared with the Large-Construct kit (Qiagen, Valencia, CA) and labeled by nick translation with Alexa Fluor 488 dUTP's (Molecular Probes, Green Island, NY) as described (Wiegant and Raap, 2001). The quality and mapping of all probes was verified by hybridization to normal metaphase spreads in combination with a commercially available centromeric probe for chromosome 17 (Openbiosystems, Lafayette, CO), before tissue analysis. Hybridization on tissue sections was performed as described previously (Wiegant and Raap, 2001). Images were taken with a Zeiss Axio Imager Z2 controlled by Axiovision software (Zeiss, Jena, Germany).

[00160] Analysis of FISH results. The FISH signals were assessed and counted manually from images with several Z stack layers. A minimum of 30 nuclei from each case were evaluated and the signals were interpreted according to guidelines described previously (Munne *et al.*, 1998) and were recorded as 2, 3, 4, or greater.

[00161] Animal studies. All animal care was in accordance with

institutional guidelines and a protocol that was approved by the University of California San Francisco Committee on Animal Research and California Pacific Medical Center Research Institute. Groups of 45-day-old female C57Bl/6 (Charles River) were inoculated by tail vein injection with 30,000 B16-F10 BPTF stables cells. $1x10^6$ 1205-Lu cells were injected by tail vain in nude mice. For subcutaneous injection $1x10^6$ 1205-Lu, B16-F10, or C8161.9 cells and $2x10^6$ U251 cells were injected, respectively.

[00162] Statistical analysis. All quantified data represents an average of at least triplicate samples or as indicated. Error bars represent standard deviation of the mean. Statistical significance was determined by the Student's *t*-test or Mann-Whitney test and two-tailed p values <0.05 were considered significant.

[00163] BPTF knockdown suppresses proliferation, *in vivo* growth, and metastatic potential of murine melanoma. The functional role of BPTF in melanoma was initially assessed using shRNA-mediated targeting in the B16-F10 murine melanoma model. BPTF expression was suppressed significantly by two different anti-BPTF shRNAs (1 and 2) as determined by quantitative real-time PCR (qRT-PCR) (Figure 1A). ShRNA-mediated suppression of BPTF expression substantially suppressed the proliferative ability of B16-F10 cells when compared to a control shRNA (Figure 1B). BPTF shRNA-expressing cells also exhibited significantly reduced invasion into Matrigel®, when compared to control shRNA-expressing cells (Figure 7A). Subcutaneous injection of BPTF shRNA-expressing cells showed a significant suppression in tumor cell growth (Figure 1C). Intravenous inoculation of BPTF shRNA in C57Bl/6 mice showed a highly significant reduction in metastatic tumor count in the lungs (Figure 1D). These results demonstrate a significant role for BPTF in the proliferative and metastatic potential of melanoma.

[00164] Due to the uncharacterized role of BPTF in cancer, cDNA microarray analyses were performed to identify the global patterns of gene expression following suppression of BPTF expression. cDNA microarray analysis was performed on B16-F10 clones stably expressing BPTF shRNA 2 *vs.* the control vector. Analysis of microarrays identified downregulation of expression of 27 genes, as well as overexpression of 1008 genes. The downregulated genes

included Bcl-xl and Ccnd2, key mediators of tumor cell proliferation and apoptosis. The differential expression of these genes (as well as Bcl-2) in B16-F10 melanoma was confirmed by qRT-PCR and by western blot analysis (Figure 1E-F).

[00165] BPTF knockdown suppresses proliferation, *in vivo* growth and metastatic potential of human melanoma. Having demonstrated a functional role for BPTF in murine melanoma cells, the role in the progression of human melanoma was analyzed. Targeting BPTF using a different shRNA (BPTF shRNA 3) than those used for the murine studies resulted in significant suppression of BPTF expression in 1205-Lu human melanoma cells, which harbor mutant BRAF (Figure 2A). Suppression of BPTF led to G1/G0 cell cycle arrest and a significant reduction in S-phase when compared to the control shRNA vector (Figure 2B). BPTF knockdown had a significant effect on the proliferative ability of melanoma cells, as evidenced by the suppression of cell survival (Figure 2C) and colony formation ability (Figure 2D, $p<0.02$), and was accompanied by significantly increased apoptotic index of 1205-Lu cells (Figure 2E, $p<0.02$). Similar effects on melanoma cell proliferation and survival were observed using two other shRNAs targeting human BPTF. Suppression of BPTF also led to a significant decrease in 1205-Lu invasiveness into Matrigel® (Figure 7B). Subcutaneous tumor cell growth in nude mice was considerably suppressed in BPTF shRNA-expressing cells when compared to control shRNA-expressing cells (Figure 2F). Finally, suppression of BPTF expression resulted in 66% reduction in the metastatic tumor burden in the lungs of nude mice upon intravenous inoculation of 1205-Lu cells (Figure 2G). These anti-tumor effects were confirmed following shRNA-mediated suppression of BPTF in the highly aggressive C8161.9 melanoma cell line, which harbors wild type BRAF (Figure 8A-E). Taken together, these studies demonstrate that BPTF plays an important role in promoting the proliferative and metastatic potential of both BRAF-wild type and mutant melanoma cell lines.

[00166] BPTF regulates the expression of BCL2, BCL-XL, CCND2, and the ERK1/2 pathway. FAC1, the truncated form of BPTF, exhibits sequence-specific DNA binding activity. Analysis of the promoter regions of BCL2 and BCL-XL indicated the presence of

possible consensus sequences for BPTF. The sensitivity DNase I treatment in the promoter regions of these genes was assessed in two human melanoma cell lines stably expressing control versus BPTF shRNA. DNase I hypersensitivity may be due to transcription factor binding or changes in nucleosome positioning or packing (Gross and Garrard, 1988). Using qPCR analysis, the DNA regions containing the putative BPTF binding sites within the BCL2 and BCL-XL were quantitated, and were found to be present at substantially reduced levels in both 1205-Lu and C8161.9 human melanoma cells expressing BPTF shRNA (Figure 3A-B). Thus, the promoter regions of BCL2 and BCL-XL exhibited increased sensitivity to DNase I treatment in human melanoma cells with reduced BPTF expression. It was then confirmed that the downregulation of these genes at the mRNA level in human melanoma cells following BPTF shRNA expression (Figure 3C-D). As these genes mediate pro-survival pathways, the proliferative signaling pathways regulated by BPTF expression were examined, and a substantial suppression in levels of pERK1/2 (Thr202/Tyr204) in BPTF shRNA-expressing melanoma cells was observed (Figure 3E-F). p90RSK (Ser380), the downstream target of pERK, was also suppressed by BPTF knockdown. BCL2 and BCL-XL, which represent additional effectors of the ERK pathway (Boucher *et al.*, 2000), were also modulated by BPTF knockdown, in addition to CCND2, in 1205-Lu and C8161.9 human melanoma cells (Figure 3E-F). Overexpression of either ERK1/2 or BCL-XL cDNA in melanoma cells stably-expressing BPTF shRNA reversed the suppression in cell survival following BPTF knockdown, indicating that the pro-proliferative function of BPTF is mediated, at least in part, by ERK1/2 and BCL-XL (Figure 9).

[00167] Conversely, overexpression of BPTF in 1205-Lu cells resulted in significantly increased proliferative capacity (Figure 10A), and was accompanied by upregulation in expression of CCND2, BCL-XL and BCL2 at the mRNA and protein levels (Figure 10 B-C).

[00168] **Quantitation of BPTF levels in melanocytic neoplasms.** Having determined functional roles for BPTF in promoting murine and human melanoma proliferation and metastasis, immunohistochemical analysis of BPTF expression was performed on a tissue microarray cohort of 311 human melanoma patients (Rangel *et al.*, 2006), and quantitated BPTF levels (Figure 11) using a digital imaging

analysis (Kashani-Sabet *et al.*, 2009). By Kaplan-Meier analysis, high levels of BPTF expression were significantly predictive of reduced distant metastasis-free survival (DMFS, p=0.03, Figure 4A) and disease-specific survival (DSS, p=0.008, Figure 4B). By multivariate Cox regression analysis, increasing BPTF expression was an independent predictor of DMFS (Table 1) and DSS (Table 2). Thus, BPTF overexpression directly correlated with the development of distant metastasis and with reduced survival in human melanoma, and was an independent predictor of survival.

[00169] **Table 1:** Multivariate Cox regression analysis of impact of various prognostic factors on distant-free survival of melanoma cohort.

Prognostic factor	Chi-square	Risk Ratio	P value
Tumor thickness	12.3	1.51	.0005
BPTF expression level	7.64	1.01	.0057
Ulceration	4.72	1.57	.03
Mitotic rate	4.35	1.05	.037
Site	1.25	1.25	.26
Sex	.1	.98	.75
Age	0.30	1.12	.59

[00170] **Table 2: Multivariate Cox regression analysis of impact of various prognostic factors on disease-specific survival of melanoma cohort.**

Prognostic factor	Chi-square	Risk Ratio	P value
Tumor thickness	12.4	1.56	.0004
BPTF expression level	7.49	1.01	.0062
Mitotic rate	5.26	1.06	.02
Ulceration	2.85	1.46	.09
Site	0.30	1.12	.58
Sex	0.01	.98	.92
Age	0.19	1.03	.66

[00171] To determine BPTF copy number in melanoma, interphase fluorescence *in situ* hybridization (FISH) was performed on 81 benign nevus samples and 77 primary melanomas. All nevi had 2 copies of the BPTF gene, whereas 64% of the melanomas had more than 2 copies (2 to 2.9 copies), 22% had more than 3 copies (3 to 3.9 copies) and 14% had more than 4 copies, (Table 3). Figure 4C-D shows representative FISH photomicrographs illustrative of low and high BPTF copy number. These findings indicate that the BPTF copy number is elevated in a significant proportion of primary melanomas, further supporting its oncogenic role.

[00172] **Table 3: BPTF copy number for Melanoma and Nevi samples.**

Nevi

Copy Number	Cases	%
2	81	100

Melanoma

Copy Number	Cases	%
2	49	64
3	17	22
4	11	14

[00173] **BPTF regulates sensitivity to selective BRAF inhibitors.** ERK1/2 is a downstream target of BRAF within the MAP kinase pathway, and is significantly suppressed following treatment with selective BRAF inhibitors (Greger *et al.*, 2012; Joseph *et al.*, 2010). Given the downregulation of activated ERK1/2 following shRNA-mediated suppression of BPTF in human melanoma cells, the

level of BPTF expression was assessed to determine the sensitivity to selective BRAF inhibitors. BPTF shRNA-expressing 1205-Lu cells were 3.2-fold more sensitive to vemurafenib treatment (Figure 5A), and 2.8-more sensitive to dabrafenib treatment (Figure 5B), when compared to control shRNA-expressing cells. Conversely, overexpression of BPTF in 1205-Lu cells significantly reduced their sensitivity to vemurafenib or dabrafenib treatment (2.5 to 3.4 fold, respectively) (Figure 5C-D). The regulation of sensitivity of human melanoma cells to selective BRAF inhibition following modulation of BPTF expression was confirmed in the BRAF-mutant expressing LOX cell line (Figure 12).

[00174] In addition, samples acquired from eight metastatic melanoma patients prior to initiation of and following progression to the selective BRAF inhibitors vemurafenib or dabrafenib were subjected to genotyping, immunohistochemical staining and FISH analysis. Five patients were treated with vemurafenib and three with dabrafenib. Following acquired resistance to targeted therapy, five patients had wild type NRAS and three had heterozygous NRAS mutation (codon-61). In a patient treated with vemurafenib, immunohistochemical analysis of BPTF expression indicated that, while BPTF expression was homogeneous in a metastatic specimen prior to BRAF inhibitor treatment, in the progressing specimen BPTF expression was heterogeneous, with one clone of cells with absent BPTF expression (suggestive of cells responding to treatment) and another clone of cells with high levels of BPTF expression (suggestive of cells resistant to treatment) (Figure 5E-F). Immunohistochemical analysis using antibodies targeting the melanocyte antigens MART1, HMB45 and tyrosinase revealed positive staining in both of these regions (Figure 5G), confirming the melanocytic lineage of these cells. TUNEL staining identified many of the cells expressing little or no BPTF expression as having undergone apoptosis. FISH analysis revealed heterogeneity in BPTF copy number in the progressing specimen (Figure 5H). In addition, there was an increase in mean BPTF copy number (from 2.6 ± 1.9 to 3.5 ± 2.5) when comparing the pre-treatment and progressing specimens. Similar findings are shown in specimens from a patient treated with vemurafenib (Figure 13). Overall, six (75%) out of the

eight patients' post-treatment tumor were characterized by heterogeneity of BPTF expression. Taken together, these results suggest that increased levels of BPTF can mediate acquired resistance to selective BRAF inhibitors in melanoma patients, and can be selected for during the development of acquired resistance to these agents.

[00175] **BPTF promotes glioblastoma progression.** Examination of whether BPTF was involved in the progression of other solid tumors was performed. As melanocytes are derived from the embryonic neural crest, and as BPTF expression is abundant in the human fetal brain (Bowser *et al.*, 1995), experiments were performed that were aimed to determine the functional role of BPTF in the progression of GBM, which is also of neuroectodermal origin. Stable suppression of BPTF expression by BPTF shRNA 3 (Figure 6A) in U251 human GBM cells resulted in significantly reduced proliferation, as determined by assays of cell survival (Figure 6B) and colony formation (Figure 6C). Suppression of BPTF expression led to a significant G1/G0 cell cycle arrest, with a concomitant suppression in the S-phase (Figure 6D, $p<0.01$), and in the induction of apoptosis (Figure 6E, $p<0.02$). BPTF suppression also significantly reduced the invasiveness of U251 cells (Figure 7C). BPTF knockdown resulted in suppression of the expression of BCL2, BCL-XL and CCND2 in GBM cells (Figure 6F-G), accompanied by a significant suppression in phosphorylated ERK1/2 and p90RSK (Figure 6G). *In vivo* studies showed a highly significant reduction in U251 tumor cell growth following subcutaneous inoculation upon BPTF suppression (Figure 6H). Similar effects were observed following suppression of BPTF cells in LN18 cells (Figure 14). Thus, these results demonstrate a functional role for BPTF in promoting glioblastoma progression by activating genes and pathways similar to those identified in melanoma cells.

[00176] Finally, the molecular subtypes of GBM in which BPTF expression is enriched were identified. Analysis of the TCGA data set revealed BPTF overexpression to occur most commonly in the pro-neural subset of GBMs (Verhaak *et al.*, 2010). In order to confirm this observation, BPTF expression levels were observed in an independent sample of 14 GBMs that were classified as either pro-neural or mesenchymal by virtue of expression of several

established classification markers (the mesenchymal markers CEBPB, CHI3L1 TWIST1, and the pro-neural markers, DLL3, OLIG2 and PDGFRA). BPTF expression (as determined by qRT-PCR analysis) was significantly higher in the pro-neural versus the mesenchymal subset of GBMs (Figure 6I).

[00177] A number of embodiments have been described herein. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of this disclosure. Accordingly, other embodiments are within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of prognosis of melanoma, comprising:
    i) obtaining a biological tissue sample from a subject;
    ii) measuring the level of BPTF in the subject's tissue sample;
    iii) comparing the level of BPTF in the subject's tissue sample with the mean level of BPTF from one or more control biological samples from benign nevi;
    iv) providing a prognosis to the subject based on the level for BPTF in comparison to the mean level of BPTF in the controls, wherein a similar or lower level of BPTF compared to the controls is indicative that the melanoma is in remission.

2. The method of claim 1, wherein the control biological samples comprise samples from the subject.

3. The method of claim 1, wherein the control biological samples comprise samples not from the subject.

4. A method of determining whether a subject has melanoma, comprising:
    i) obtaining a biological tissue sample from a subject;
    ii) measuring the level of BPTF in the subject's tissue sample;
    iii) comparing the level of BPTF in the subject's tissue sample with the mean level of BPTF from one or more control biological samples from benign nevi; and
    iv) determining whether the subject has melanoma based on having a significantly higher level for BPTF in comparison to the mean levels for BPTF in the controls.

5. The method of claim 4, wherein the control biological samples comprise samples from the subject.

6. The method of claim 4, wherein the control biological samples comprise samples not from the subject.

7. A method of determining whether melanoma in a subject is progressing or in remission, comprising:

    i) obtaining a biological tissue sample comprising melanoma from a subject at a first time point;

    ii) measuring the level of BPTF in the subject's tissue sample from the first time point;

    iii) obtaining a biological tissue sample comprising melanoma from the subject at a second time point;

    iv) measuring the level of BPTF in the subject's tissue sample from the second time point;

    v) comparing the levels of BPTF from the first time point with the levels from the second time point; and

    vi) determining whether melanoma is progressing or is in recovery based upon the change in levels of BPTF from the two time points wherein an increase in BPTF levels between the first time point and the second time point indicates melanoma is progressing.

8. The method of claim 7, wherein the subject has been treated with an anti-cancer therapeutic agent following the step of obtaining the sample at the first time point and before the second sample is obtained at the second time point.

9. The method of claim 7, wherein increased BPTF levels further indicates that a BPTF inhibitory agent may be administered to the subject.

10. A method of providing a prognosis of the survival rate of a subject who has melanoma, comprising:

    i) obtaining a biological tissue sample comprising melanoma from a subject at a first time point;

    ii) measuring the level of BPTF in the subject's tissue sample from the first time point;

iii) obtaining a biological tissue sample comprising melanoma from a subject at a second time point;

iv) measuring the level of BPTF in the subject's tissue sample from the second time point;

v) comparing the levels of BPTF from the first time point with the levels from the second time point; and

vi) providing a prognosis of the subject's survival rate based upon the change in levels of BPTF from the two time points, wherein an increase in BPTF levels between the first time point and the second time point indicates a poor survival rate for the subject, and wherein a decrease in BPTF levels between the first time point and the second time point indicates a better survival rate for the subject.

11. Use of an effective amount of an inhibitory BPTF nucleic acid, for treating melanoma or breast cancer in a subject, wherein the inhibitory BPTF nucleic acid is an shRNA having a sequence selected from the group consisting of: SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5, wherein T can be U.

12. Use of an inhibitory BPTF nucleic acid, for the manufacture of a medicament for treating melanoma or breast cancer in a subject, wherein the inhibitory BPTF nucleic acid is an shRNA having a sequence selected from the group consisting of: SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5, wherein T can be U.

13. The use of claim 11 or 12, wherein the shRNA sequence comprises one or more modified base.

14. The use of any one of claims 11 to 13, wherein inhibiting the expression of BPTF results in inhibiting or preventing the proliferation or migration of cancer cells.

15. The use of any one of claims 11 to 13, wherein the inhibitory BPTF nucleic acid is for use in combination with one or more additional anti-cancer therapeutic agents.

16. The use of claim 15, wherein the one or more additional therapeutic agents are selected from the group consisting of platinum analogs, alkylating agents, alkyl sulfonates, androgens, anti-adrenals, anti-androgens, antibiotics, anti-estrogens, aromatase inhibiting 4(5)-imidazoles, anti-metabolites, folic acid analogues, ethylenimines and methylamelamines, folic acid replenishers, nitrogen mustards, nitrosureas, purine analogs, pyrimidine analogs, topoisomerase inhibitors, thymidylate synthase inhibitors, anti-cancer antibodies, chemotherapeutics, de-methylation agents, and targeted therapeutic agents.

17. The use of any one of claims 11 to 13, wherein the inhibitory BPTF nucleic acid is a vector comprising the inhibitory BPTF nucleic acid.

18. The use of claim 17, wherein the vector comprises an expression vector.

19. The use of claim 17, wherein the vector comprises a replication competent retroviral vector.

20. A composition comprising a BPTF inhibitor and a first-line anti-cancer therapeutic, wherein the BPTF inhibitor is an inhibitory BPTF nucleic acid having a sequence selected from the group consisting of: SEQ ID NO. 3, SEQ ID NO. 4, and SEQ ID NO. 5, wherein T can be U.

21. The composition of claim 20, wherein the nucleic acid sequence comprises one or more modified base.

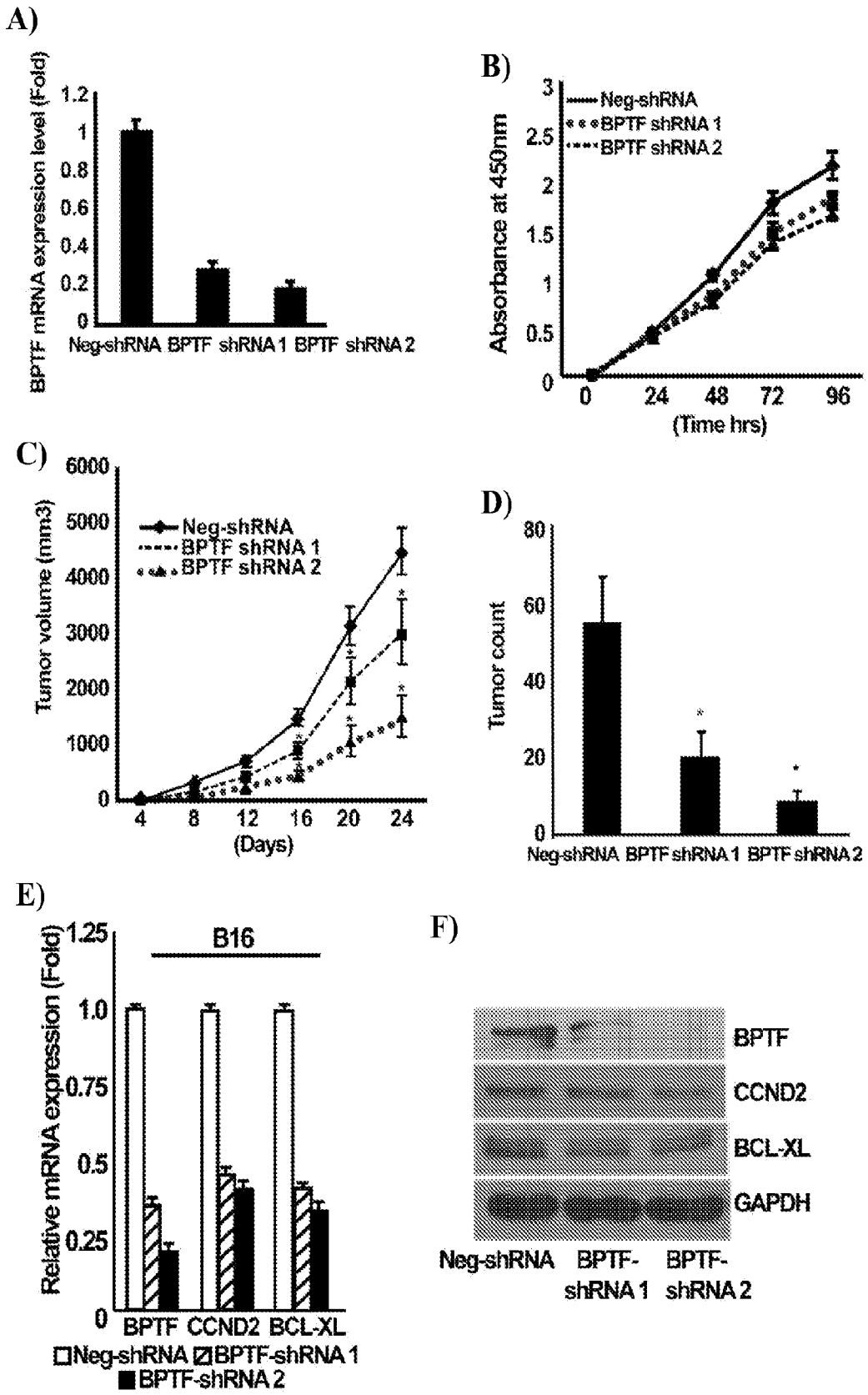

A)
BPTF mRNA expression level (Fold)
1.2
1
0.8
0.6
0.4
0.2
0
Neg-shRNA
BPTF shRNA 1
BPTF shRNA 2
B)
Absorbance at 450nm
3
2.5
2
1.5
1
0.5
0
Neg-shRNA
BPTF shRNA 1
BPTF shRNA 2
0
24
48
72
96
(Time hrs)
C)
Tumor volume (mm3)
6000
5000
4000
3000
2000
1000
0
Neg-shRNA
BPTF shRNA 1
BPTF shRNA 2
4
8
12
16
20
24
(Days)
D)
Tumor count
80
60
40
20
0
Neg-shRNA
BPTF shRNA 1
BPTF shRNA 2
E)
B16
Relative mRNA expression (Fold)
1.25
1.0
0.75
0.5
0.25
0
BPTF
CCND2
BCL-XL
Neg-shRNA
BPTF-shRNA 1
BPTF-shRNA 2
F)
BPTF
CCND2
BCL-XL
GAPDH
Neg-shRNA
BPTF-shRNA 1
BPTF-shRNA 2

FIGURE 1A-F

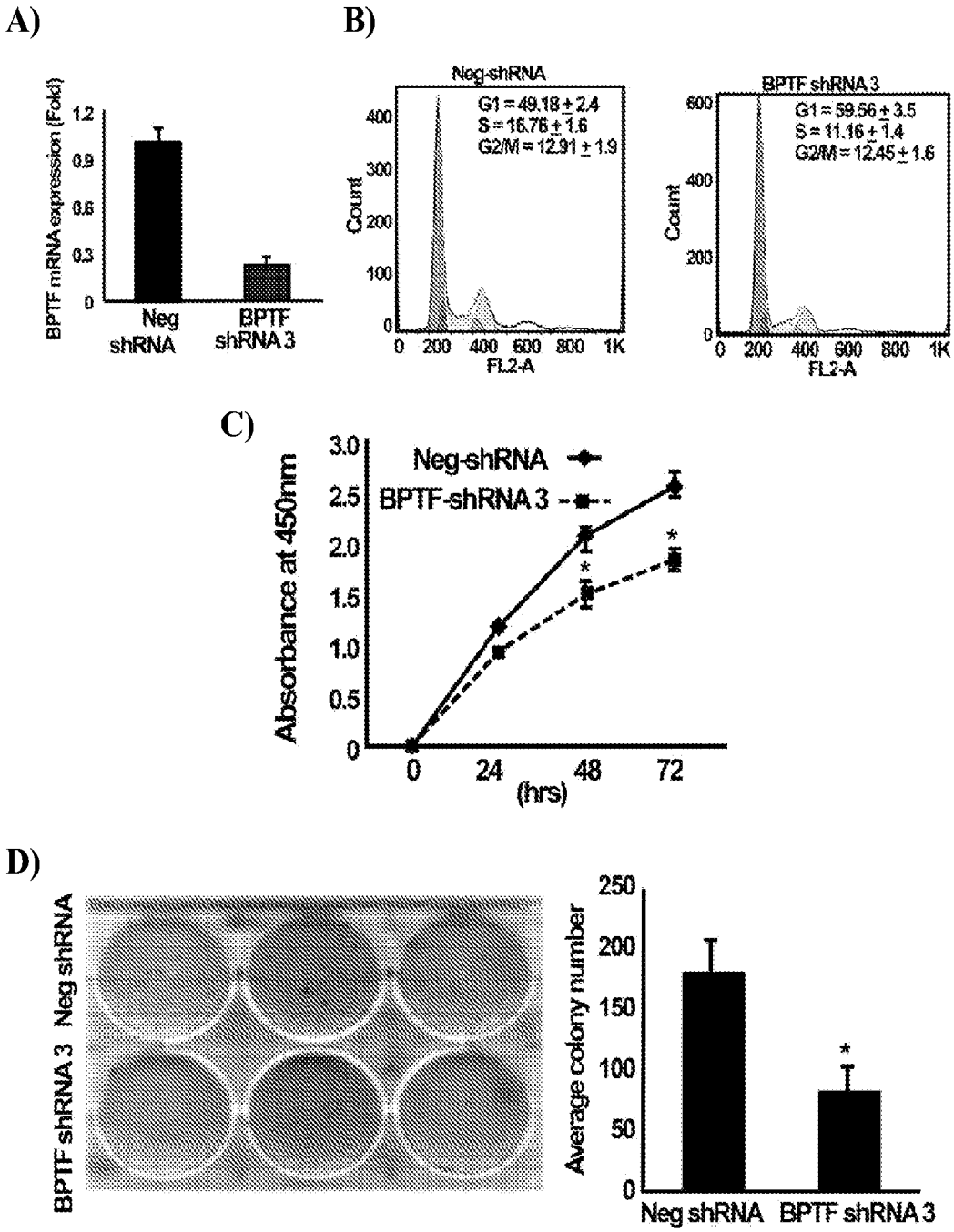

A)
B)
C)
D)
BPTF mRNA expression (Fold)
1.2
0.9
0.6
0.3
0
Neg shRNA
BPTF shRNA 3
Neg-shRNA
G1 = 49.18 ± 2.4
S = 16.76 ± 1.6
G2/M = 12.91 ± 1.9
Count
FL2-A
BPTF shRNA 3
G1 = 59.56 ± 3.5
S = 11.16 ± 1.4
G2/M = 12.45 ± 1.6
Absorbance at 450nm
Neg-shRNA
BPTF-shRNA 3
(hrs)
BPTF shRNA 3
Neg shRNA
Average colony number
Neg shRNA
BPTF shRNA 3

Figure 2A-D

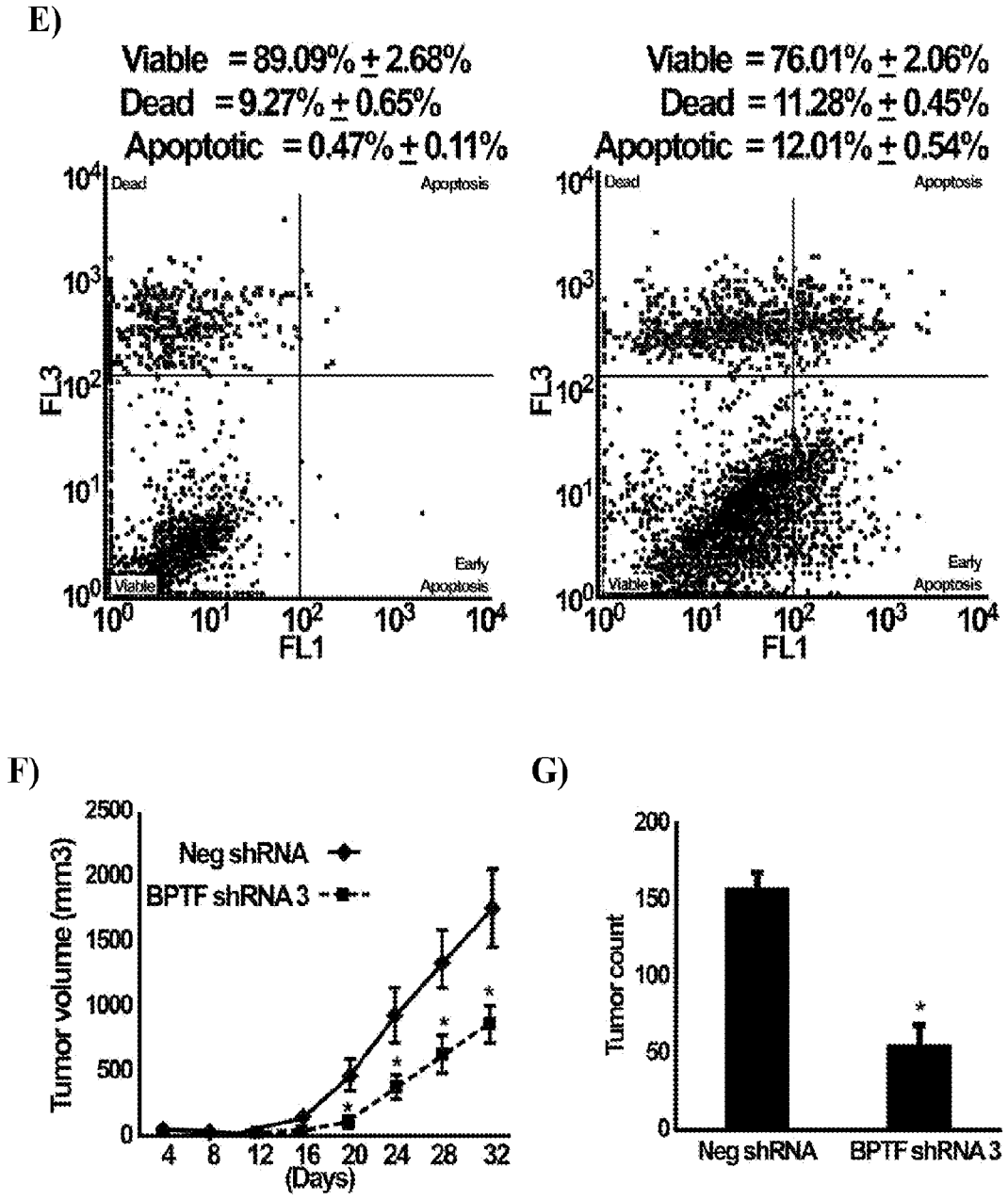

E)
Viable = 89.09% ± 2.68%
Dead = 9.27% ± 0.65%
Apoptotic = 0.47% ± 0.11%
Viable = 76.01% ± 2.06%
Dead = 11.28% ± 0.45%
Apoptotic = 12.01% ± 0.54%
Dead
Apoptosis
Viable
Early Apoptosis
FL3
FL1
F)
Tumor volume (mm3)
Neg shRNA
BPTF shRNA 3
(Days)
G)
Tumor count
Neg shRNA
BPTF shRNA 3

Figure 2E-G

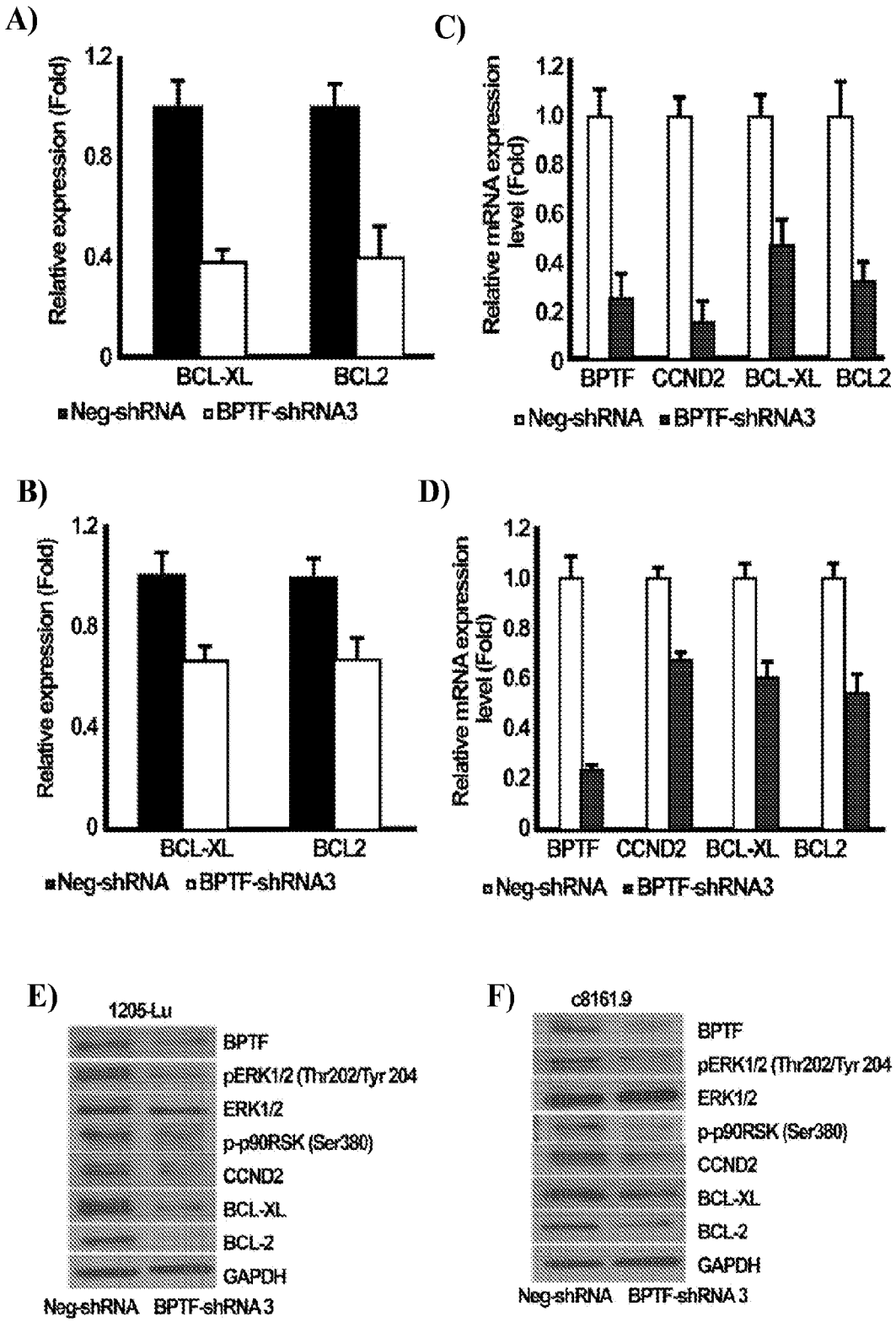

A)
Relative expression (Fold)
1.2
0.8
0.4
0
BCL-XL
BCL2
Neg-shRNA
BPTF-shRNA3
C)
Relative mRNA expression level (Fold)
1.2
1.0
0.8
0.6
0.4
0.2
0
BPTF
CCND2
BCL-XL
BCL2
Neg-shRNA
BPTF-shRNA3
B)
Relative expression (Fold)
1.2
0.8
0.4
0
BCL-XL
BCL2
Neg-shRNA
BPTF-shRNA3
D)
Relative mRNA expression level (Fold)
1.2
1.0
0.8
0.6
0.4
0.2
0
BPTF
CCND2
BCL-XL
BCL2
Neg-shRNA
BPTF-shRNA3
E)
1205-Lu
BPTF
pERK1/2 (Thr202/Tyr 204
ERK1/2
p-p90RSK (Ser380)
CCND2
BCL-XL
BCL-2
GAPDH
Neg-shRNA
BPTF-shRNA 3
F)
c8161.9
BPTF
pERK1/2 (Thr202/Tyr 204
ERK1/2
p-p90RSK (Ser380)
CCND2
BCL-XL
BCL-2
GAPDH
Neg-shRNA
BPTF-shRNA 3

Figure 3A-F

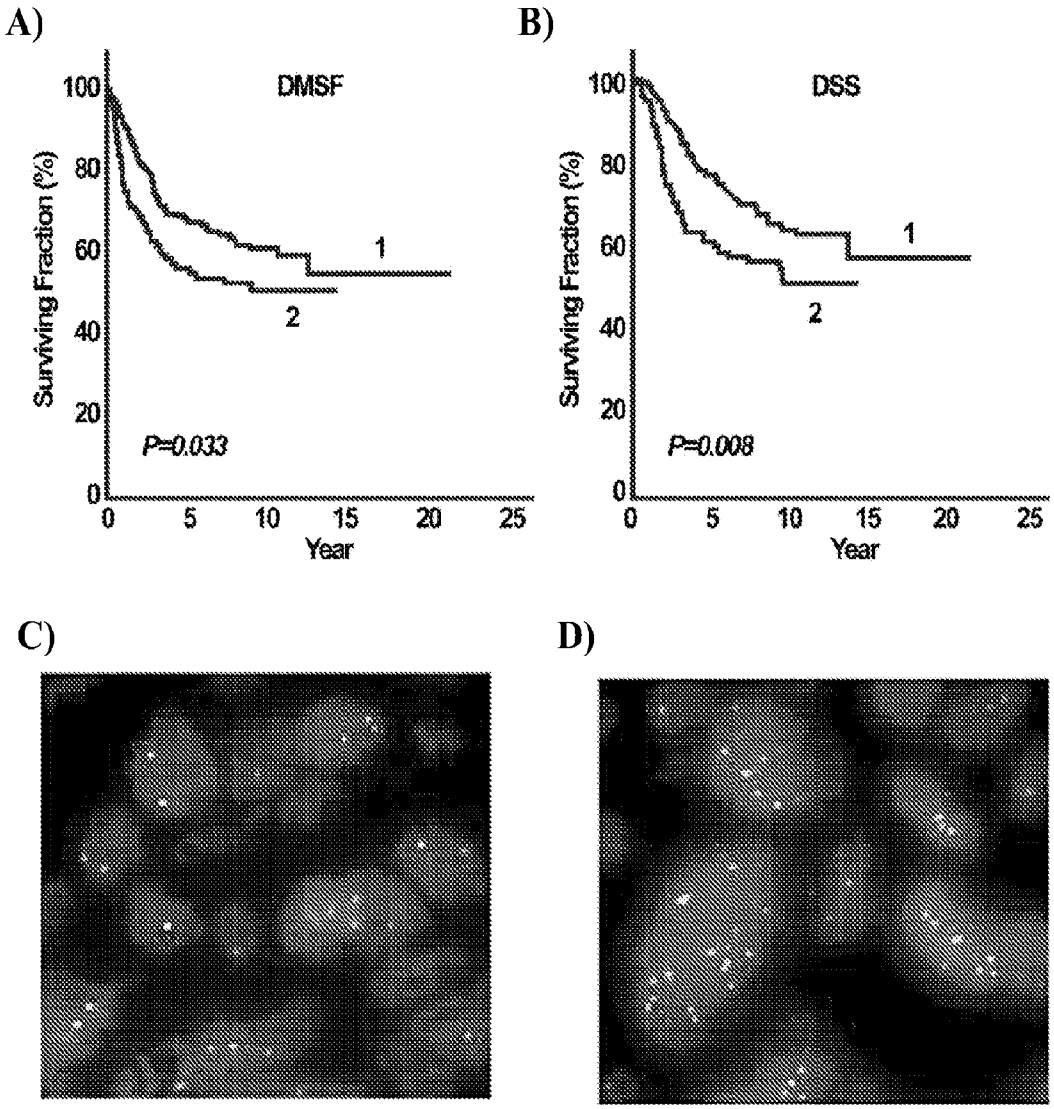

A)
DMSF
Surviving Fraction (%)
100
80
60
40
20
0
1
2
P=0.033
0
5
10
15
20
25
Year
B)
DSS
Surviving Fraction (%)
100
80
60
40
20
0
1
2
P=0.008
0
5
10
15
20
25
Year
C)
D)

Figure 4A-D

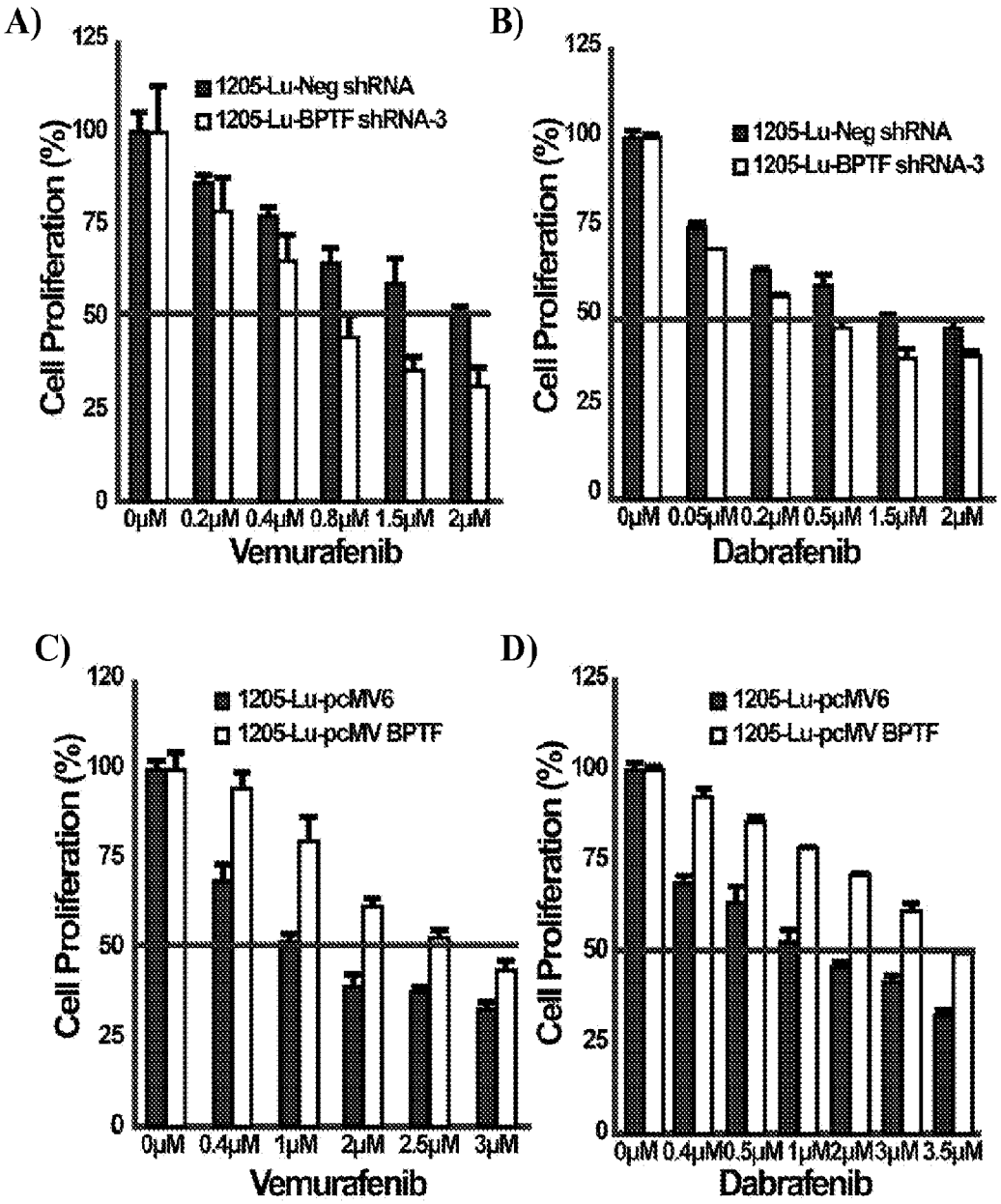

A)
125
100
75
50
25
0
Cell Proliferation (%)
1205-Lu-Neg shRNA
1205-Lu-BPTF shRNA-3
0μM 0.2μM 0.4μM 0.8μM 1.5μM 2μM
Vemurafenib
B)
125
100
75
50
25
0
Cell Proliferation (%)
1205-Lu-Neg shRNA
1205-Lu-BPTF shRNA-3
0μM 0.05μM 0.2μM 0.5μM 1.5μM 2μM
Dabrafenib
C)
120
100
75
50
25
0
Cell Proliferation (%)
1205-Lu-pcMV6
1205-Lu-pcMV BPTF
0μM 0.4μM 1μM 2μM 2.5μM 3μM
Vemurafenib
D)
125
100
75
50
25
0
Cell Proliferation (%)
1205-Lu-pcMV6
1205-Lu-pcMV BPTF
0μM 0.4μM 0.5μM 1μM 2μM 3μM 3.5μM
Dabrafenib

Figure 5A-D

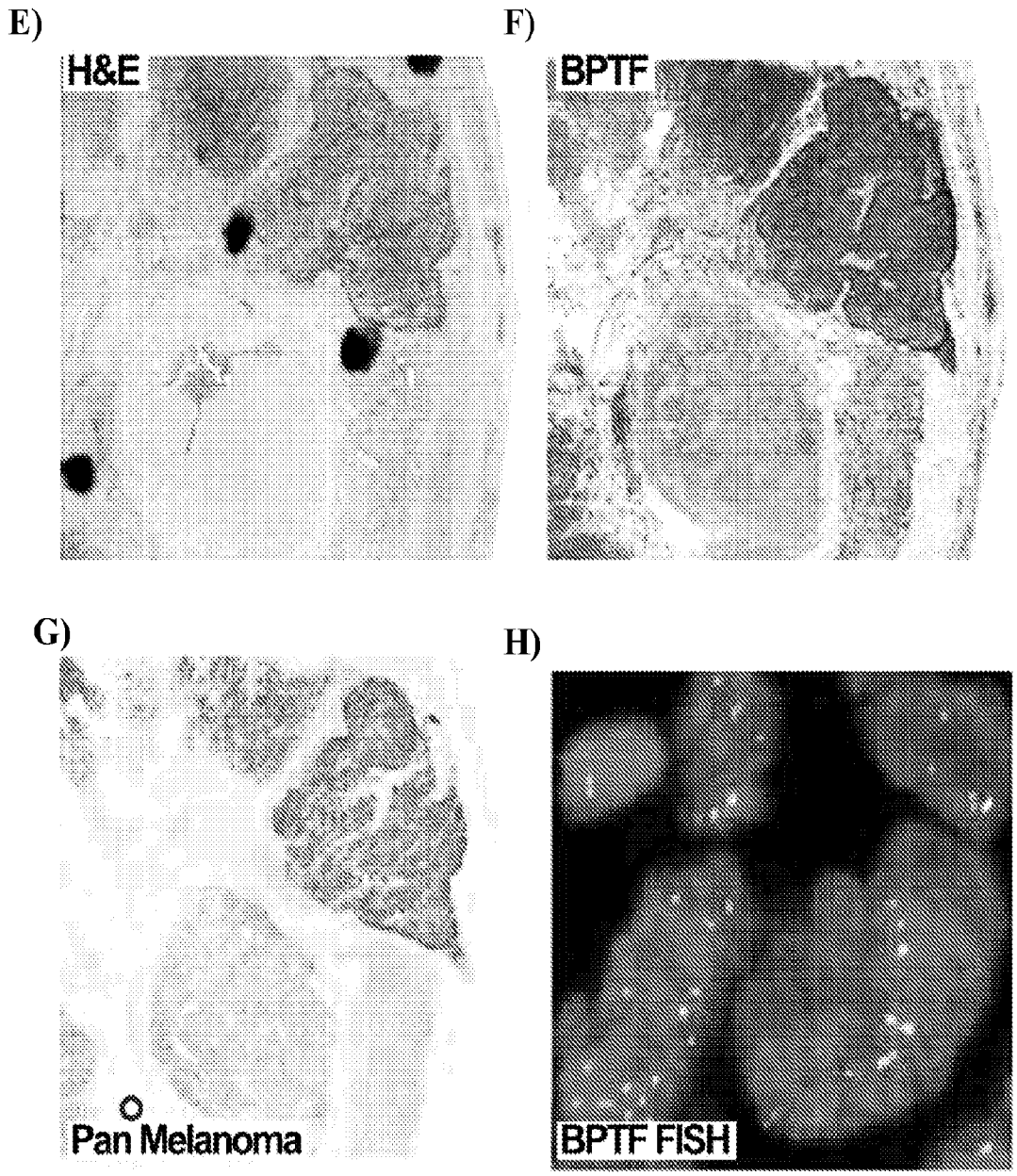


Figure 5E-H

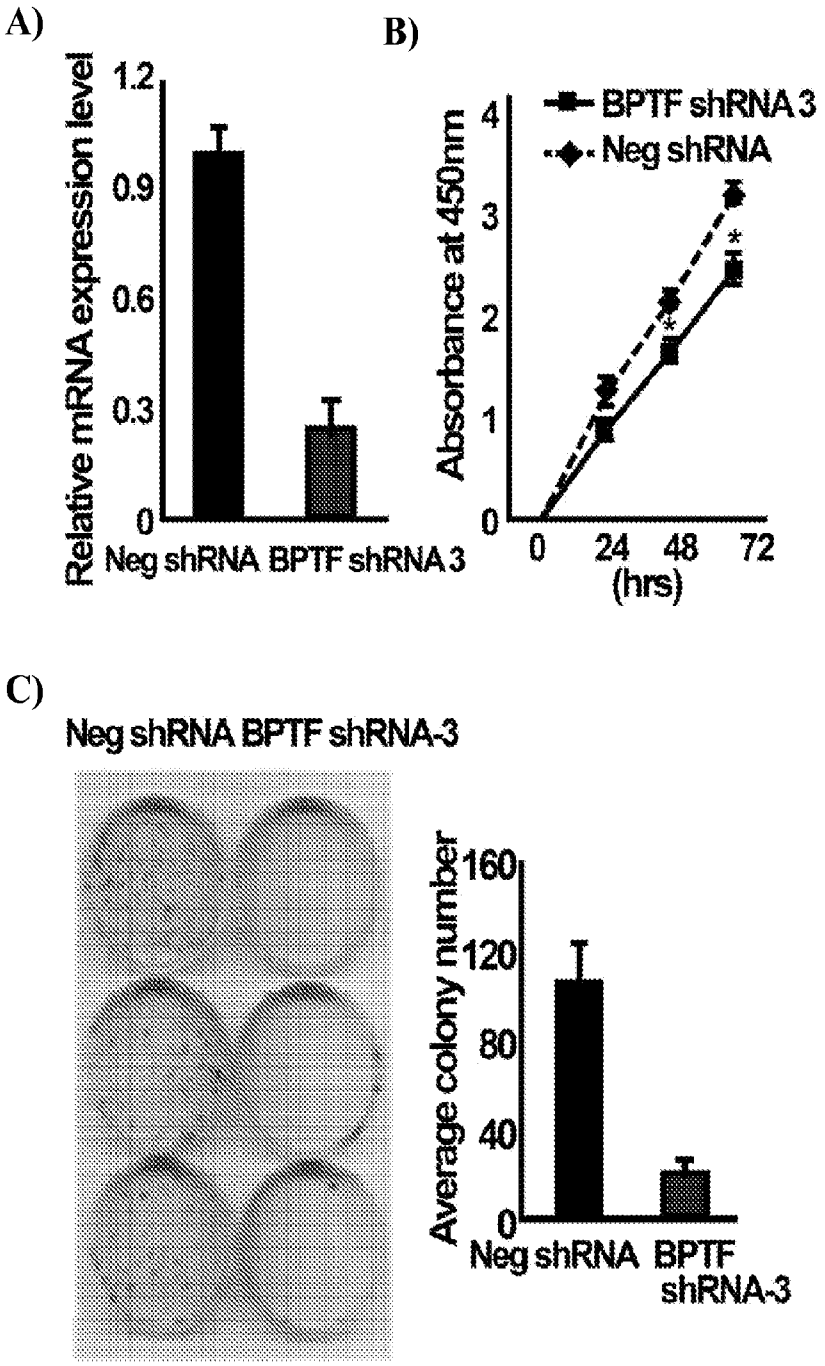
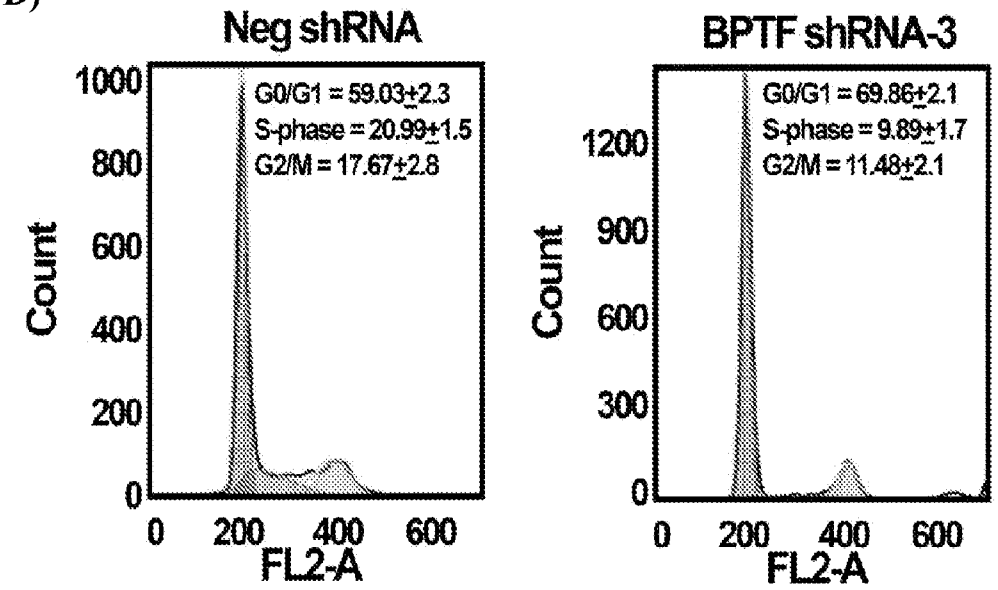

A)
B)
Relative mRNA expression level
1.2
0.9
0.6
0.3
0
Neg shRNA
BPTF shRNA 3
Absorbance at 450nm
4
3
2
1
0
0
24
48
72
(hrs)
BPTF shRNA 3
Neg shRNA
*
C)
Neg shRNA
BPTF shRNA-3
Average colony number
160
120
80
40
0
Neg shRNA
BPTF shRNA-3

Figure 6A-C

D)



E)

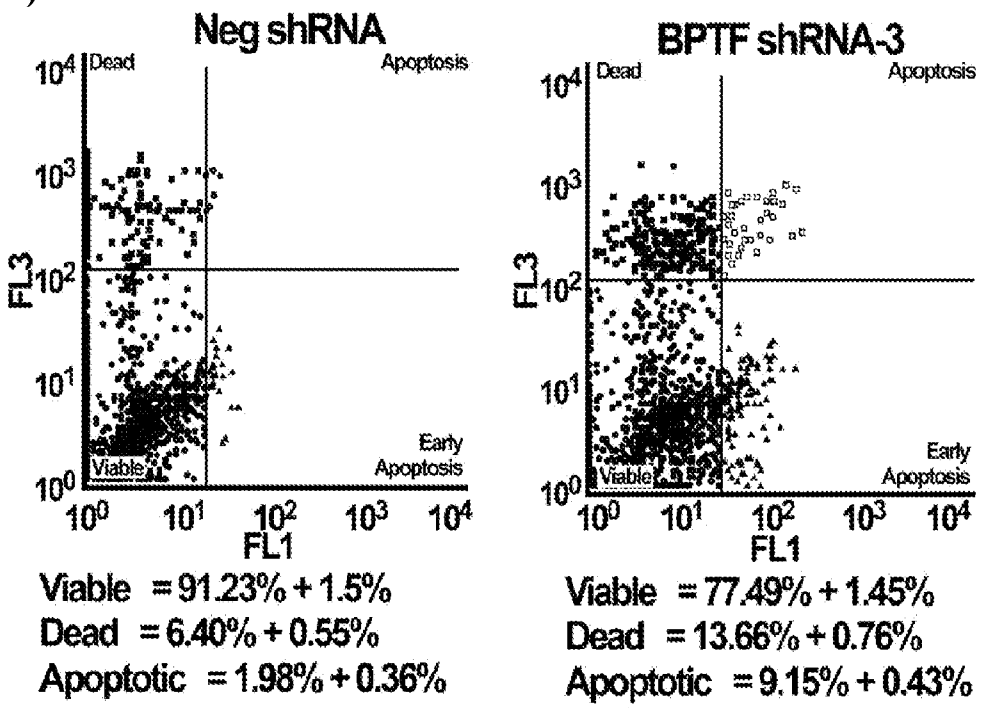
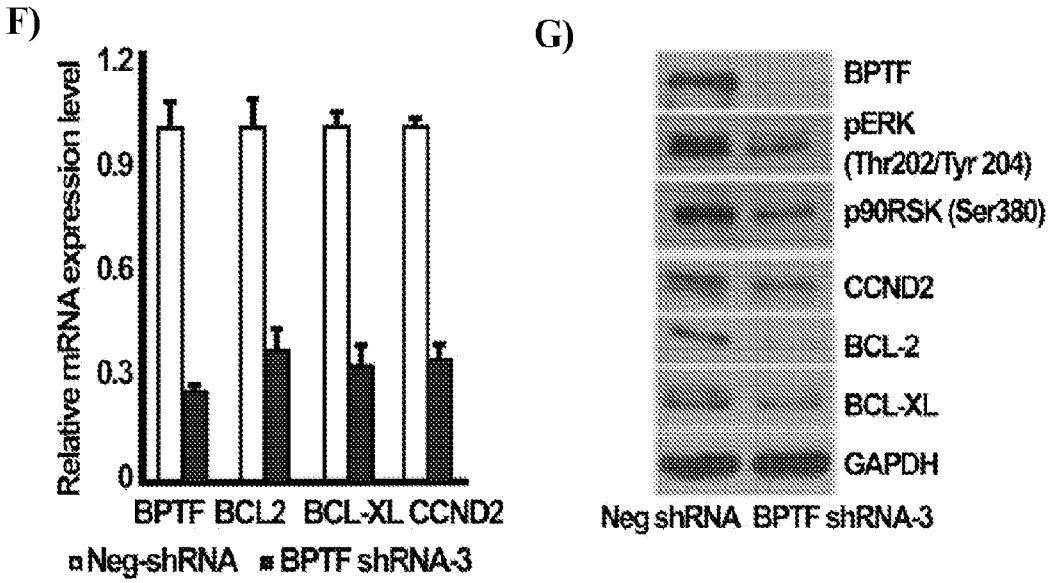


Figure 6D-E


F)
Relative mRNA expression level
1.2
0.9
0.6
0.3
0
BPTF BCL2 BCL-XL CCND2
Neg-shRNA BPTF shRNA-3
G)
BPTF
pERK
(Thr202/Tyr 204)
p90RSK (Ser380)
CCND2
BCL-2
BCL-XL
GAPDH
Neg shRNA BPTF shRNA-3

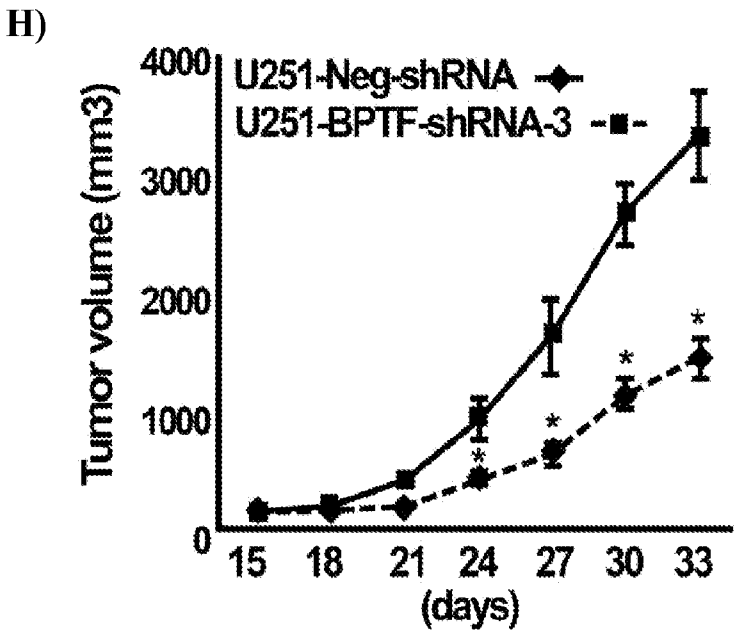

H)
Tumor volume (mm3)
4000
3000
2000
1000
0
U251-Neg-shRNA
U251-BPTF-shRNA-3
15 18 21 24 27 30 33
(days)

Figure 6F-H

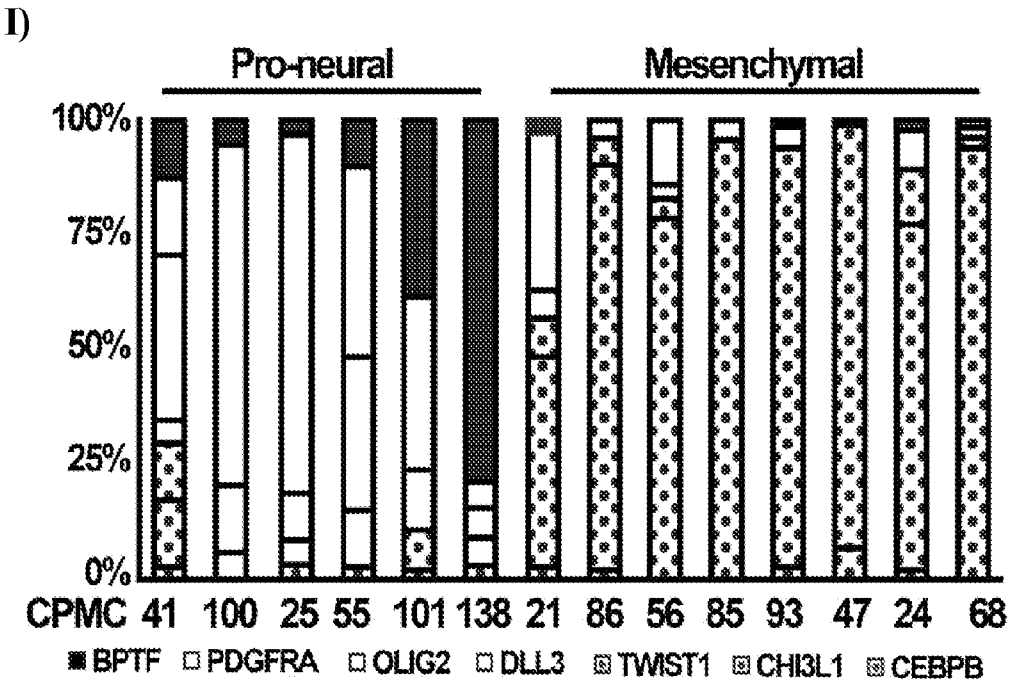


Figure 6I

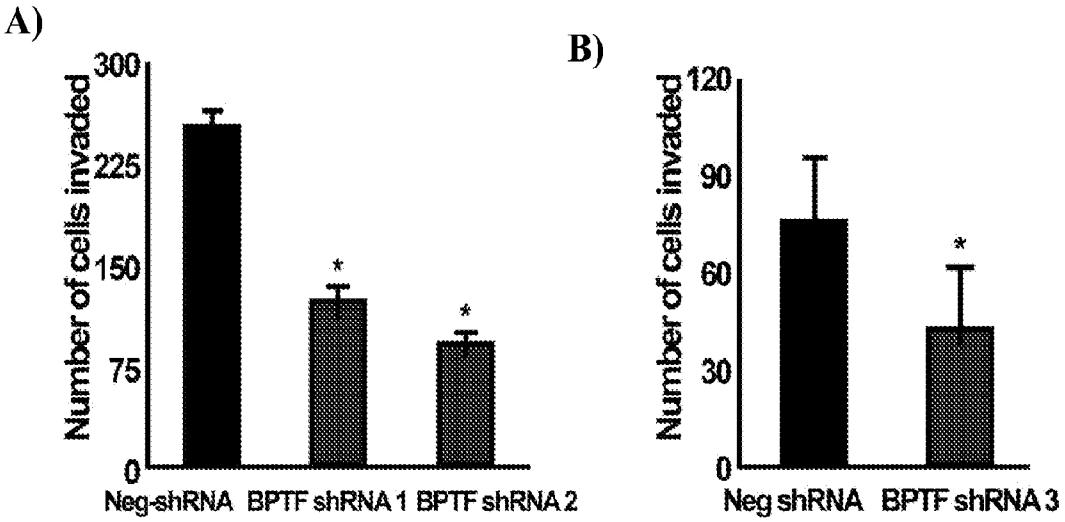


Figure 7A-B

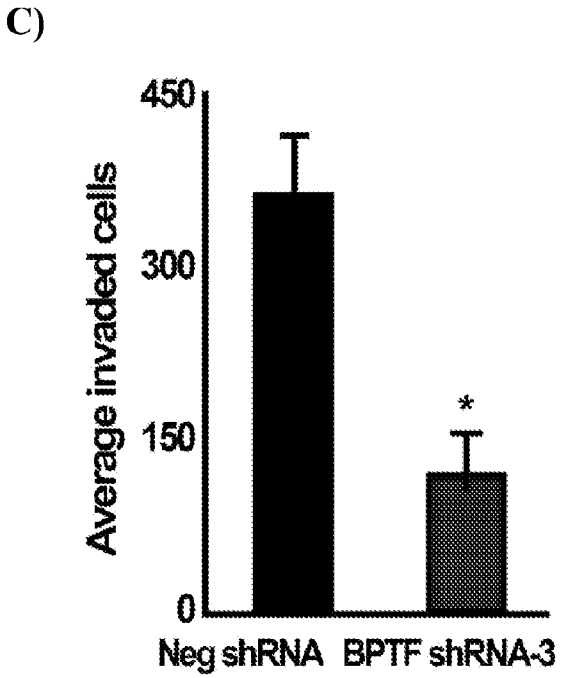


Figure 7C

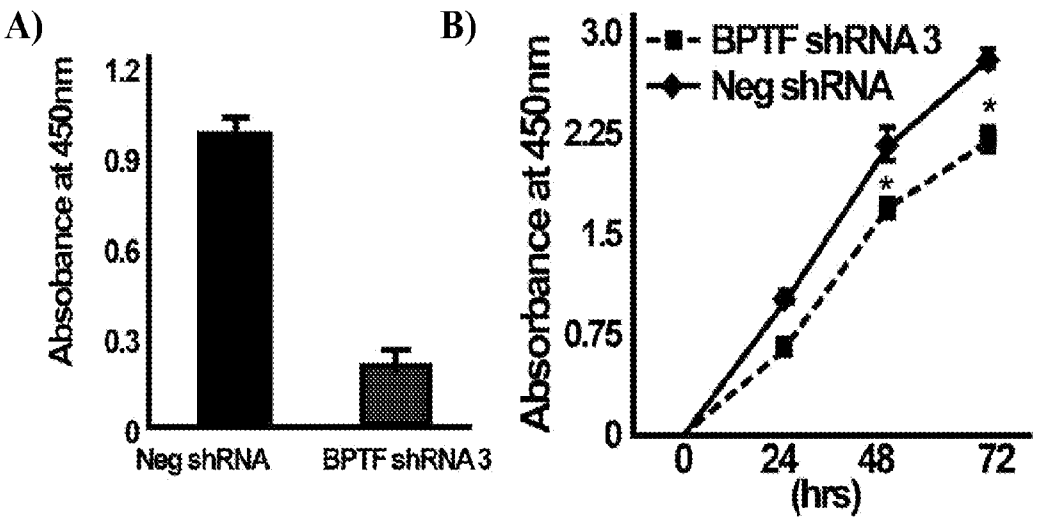


Figure 8A-B

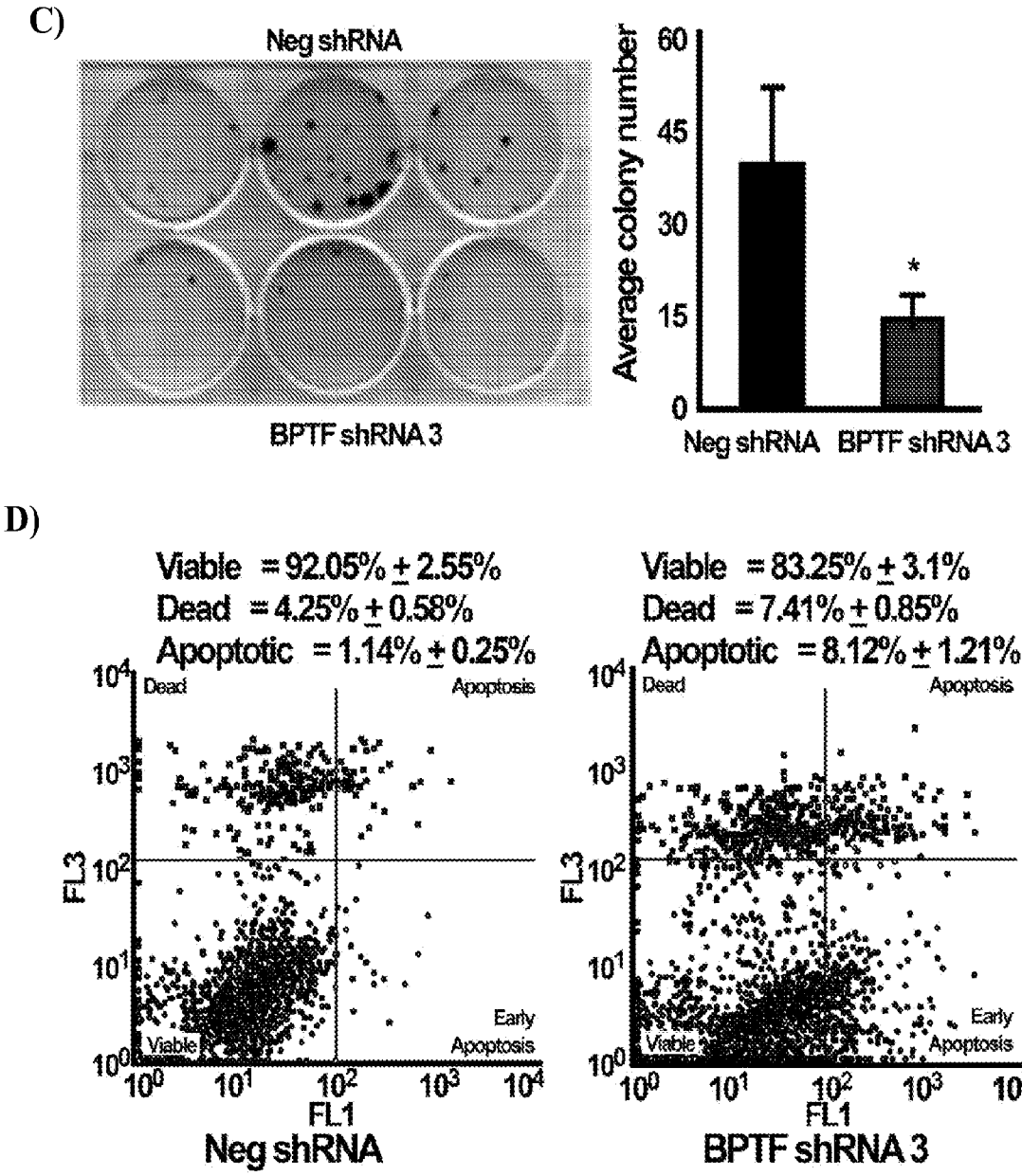

C)
Neg shRNA
BPTF shRNA 3
Average colony number
60
45
30
15
0
*
Neg shRNA
BPTF shRNA 3
D)
Viable = 92.05% ± 2.55%
Dead = 4.25% ± 0.58%
Apoptotic = 1.14% ± 0.25%
Viable = 83.25% ± 3.1%
Dead = 7.41% ± 0.85%
Apoptotic = 8.12% ± 1.21%
Dead
Apoptosis
Early Apoptosis
Viable
FL3
FL1
Neg shRNA
BPTF shRNA 3

Figure 8C-D

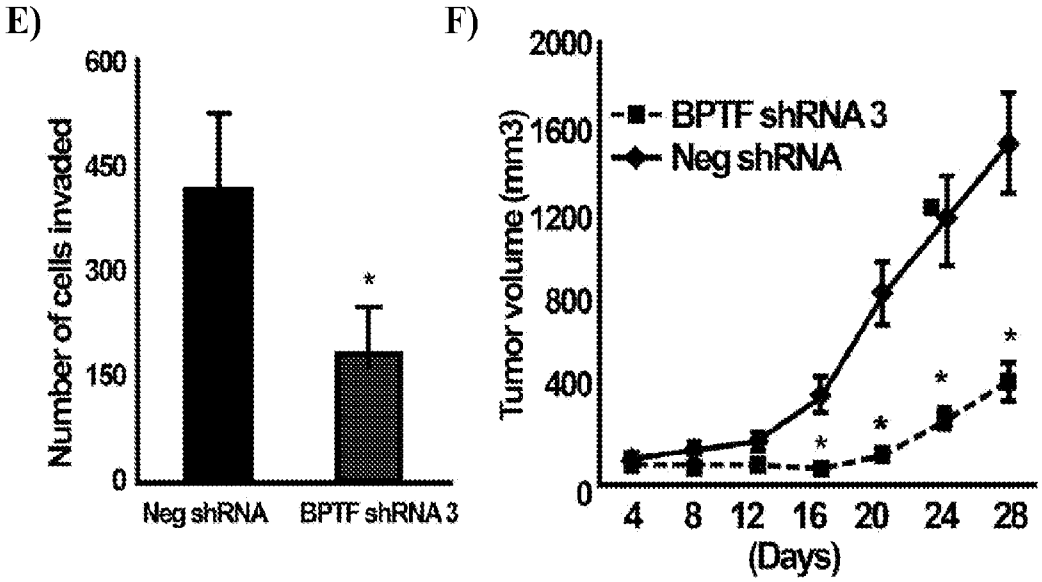


Figure 8E-F

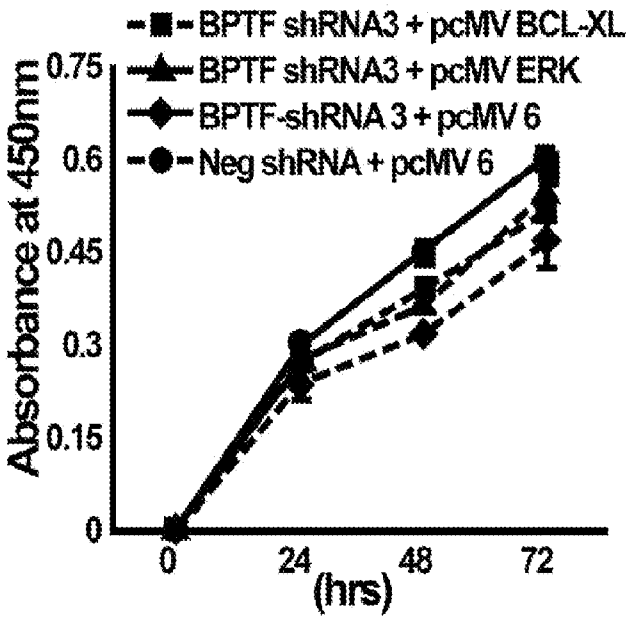


FIGURE 9

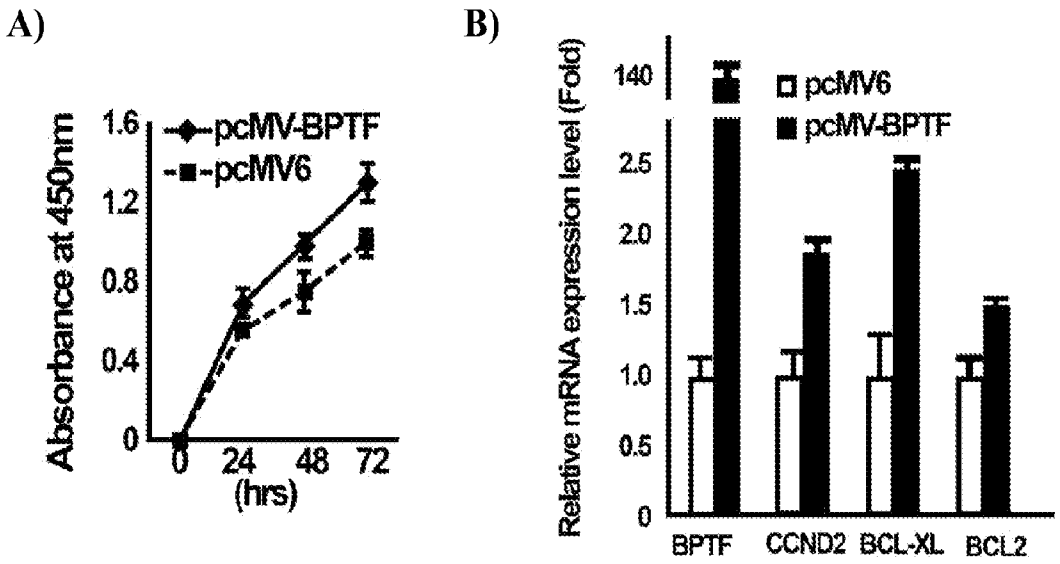


Figure 10A-B

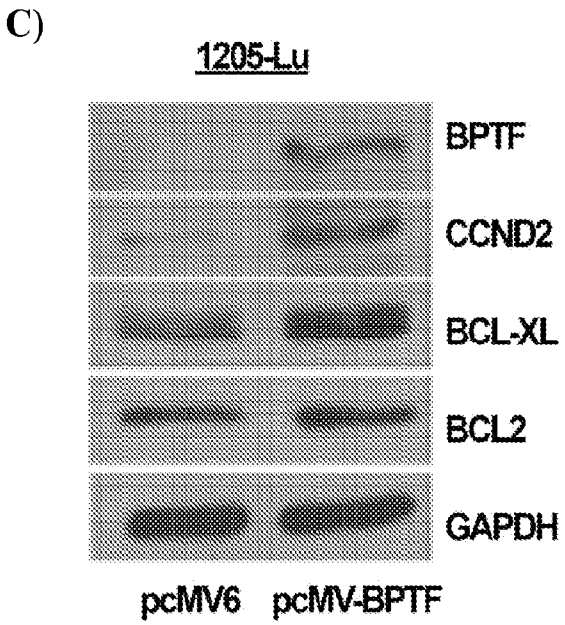


FIGURE 10C

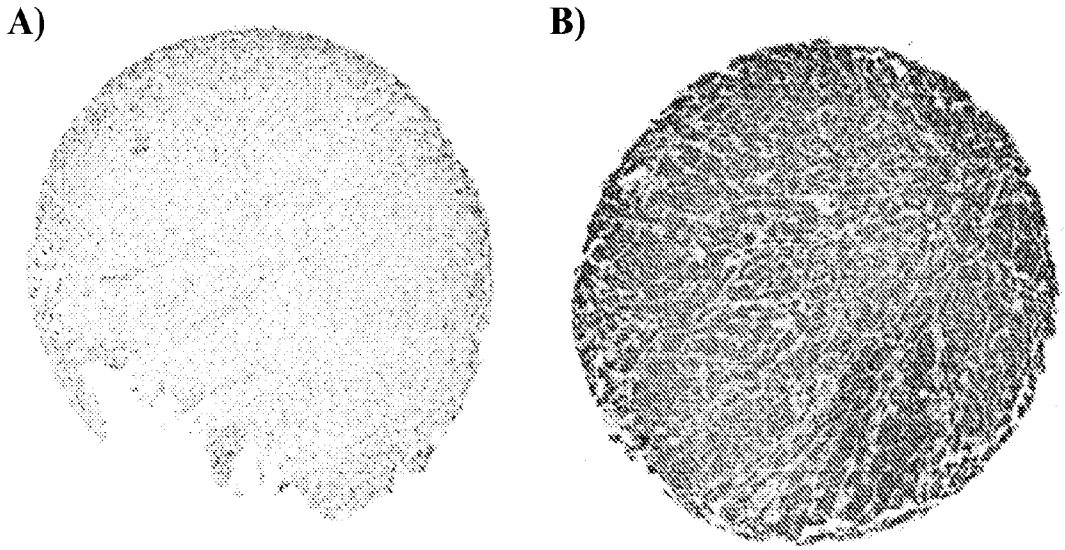


FIGURE 11A-B

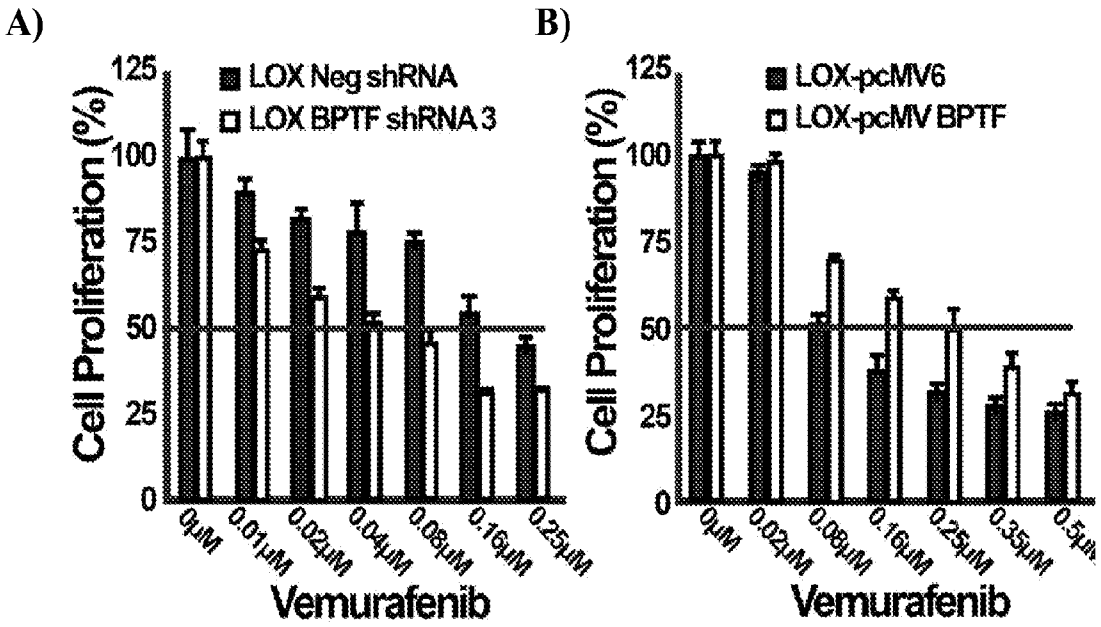


FIGURE 12A-B

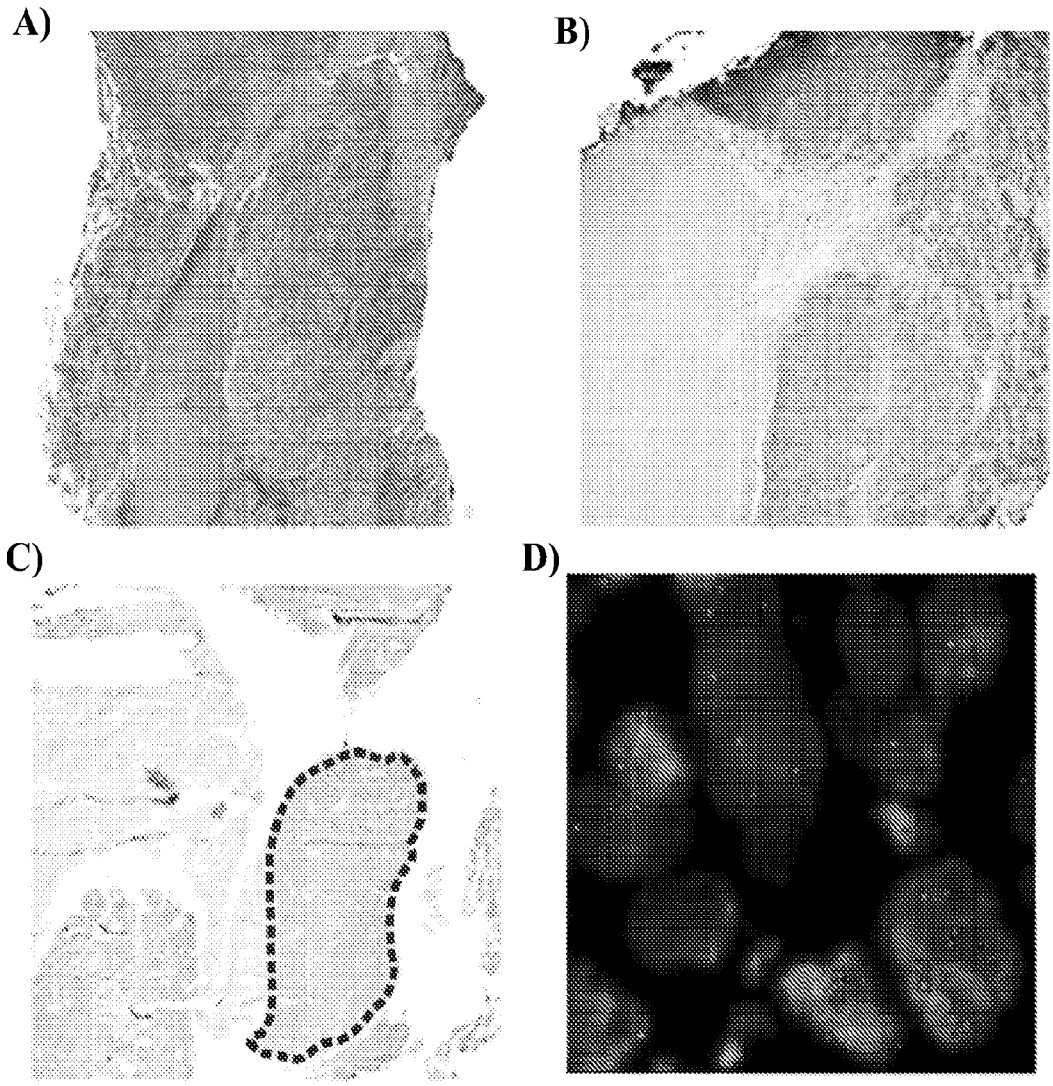


FIGURE 13A-D

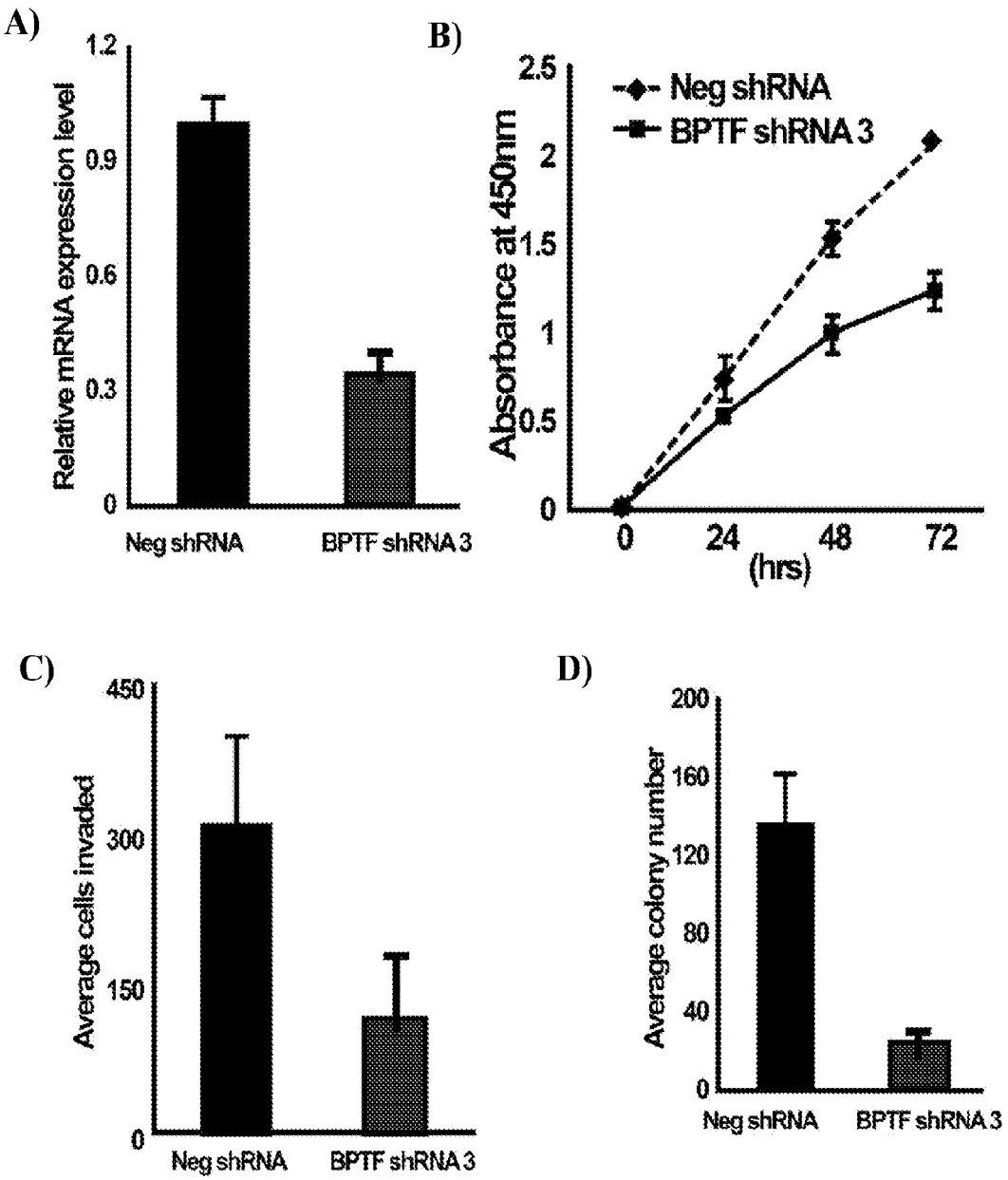

A)
Relative mRNA expression level
1.2
0.9
0.6
0.3
0
Neg shRNA
BPTF shRNA 3
B)
Absorbance at 450nm
2.5
2
1.5
1
0.5
0
Neg shRNA
BPTF shRNA 3
0
24
48
72
(hrs)
C)
Average cells invaded
450
300
150
0
Neg shRNA
BPTF shRNA 3
D)
Average colony number
200
160
120
80
40
0
Neg shRNA
BPTF shRNA 3

Figure 14A-D

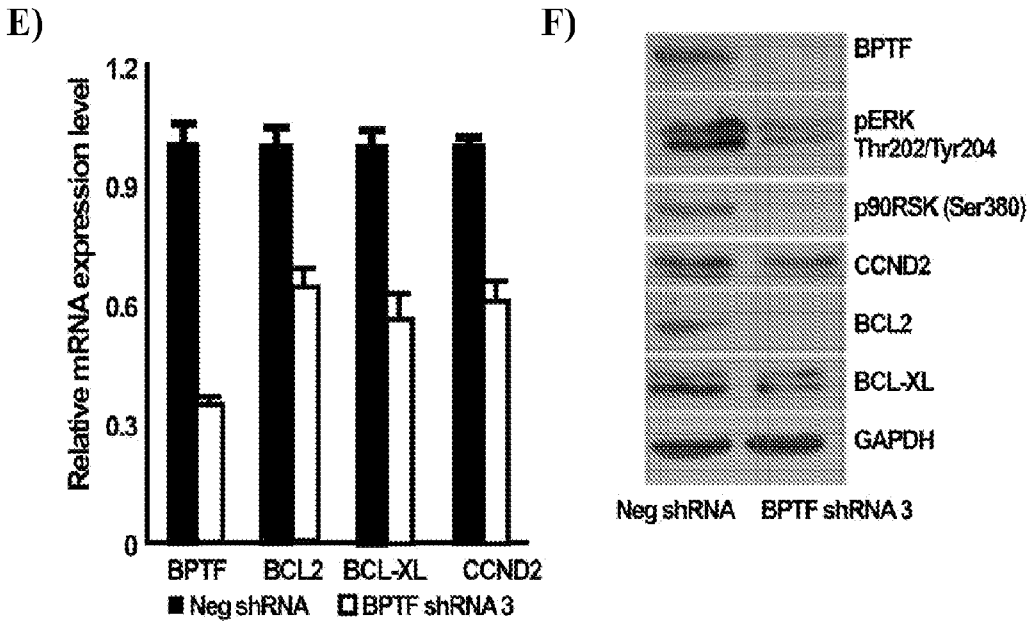


FIGURE 14E-F

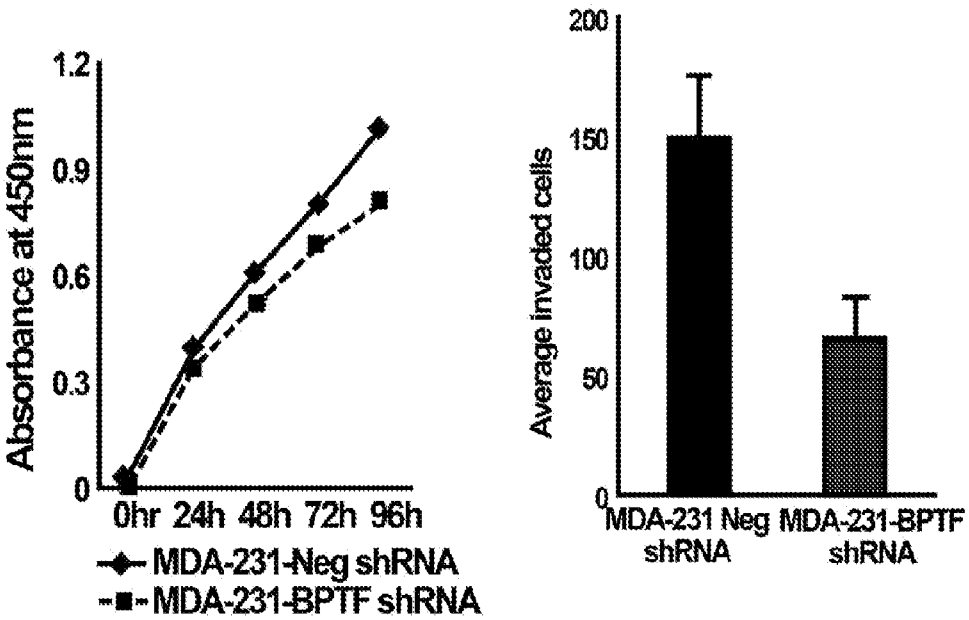


FIGURE 15

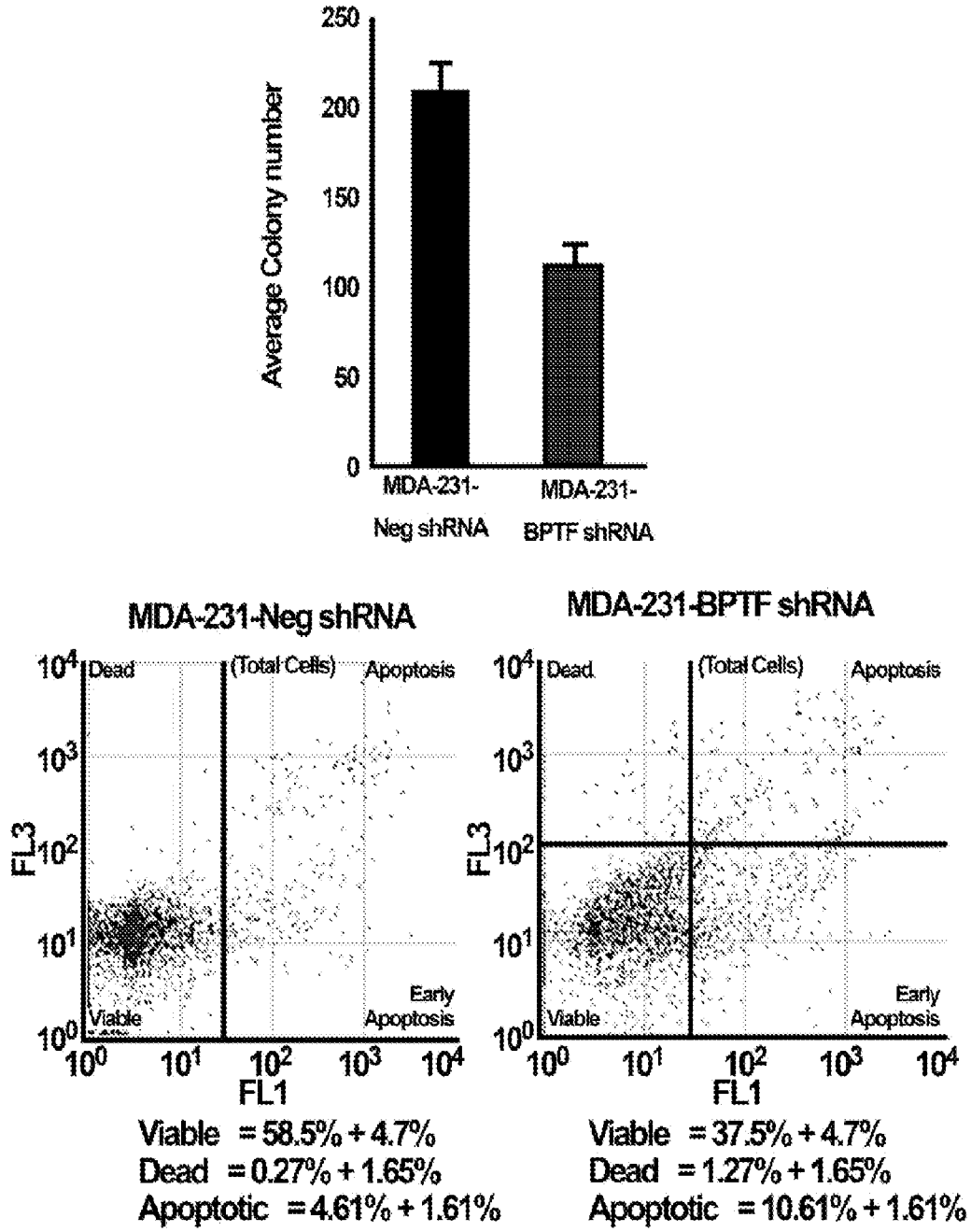

Average Colony number
250
200
150
100
50
0
MDA-231-Neg shRNA
MDA-231-BPTF shRNA
MDA-231-Neg shRNA
MDA-231-BPTF shRNA
Dead
(Total Cells)
Apoptosis
Viable
Early Apoptosis
FL3
FL1
Viable = 58.5% + 4.7%
Dead = 0.27% + 1.65%
Apoptotic = 4.61% + 1.61%
Viable = 37.5% + 4.7%
Dead = 1.27% + 1.65%
Apoptotic = 10.61% + 1.61%

FIGURE 16

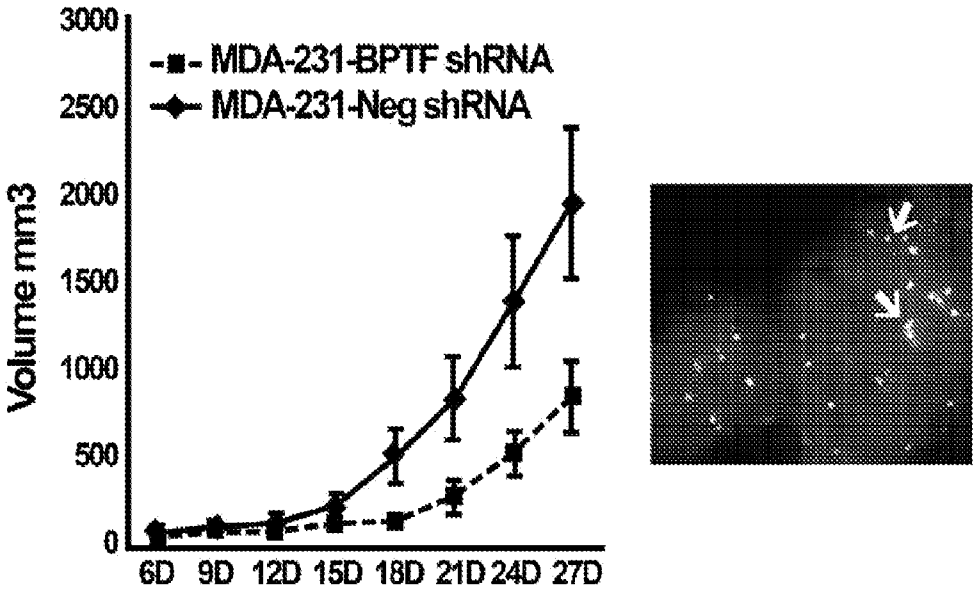
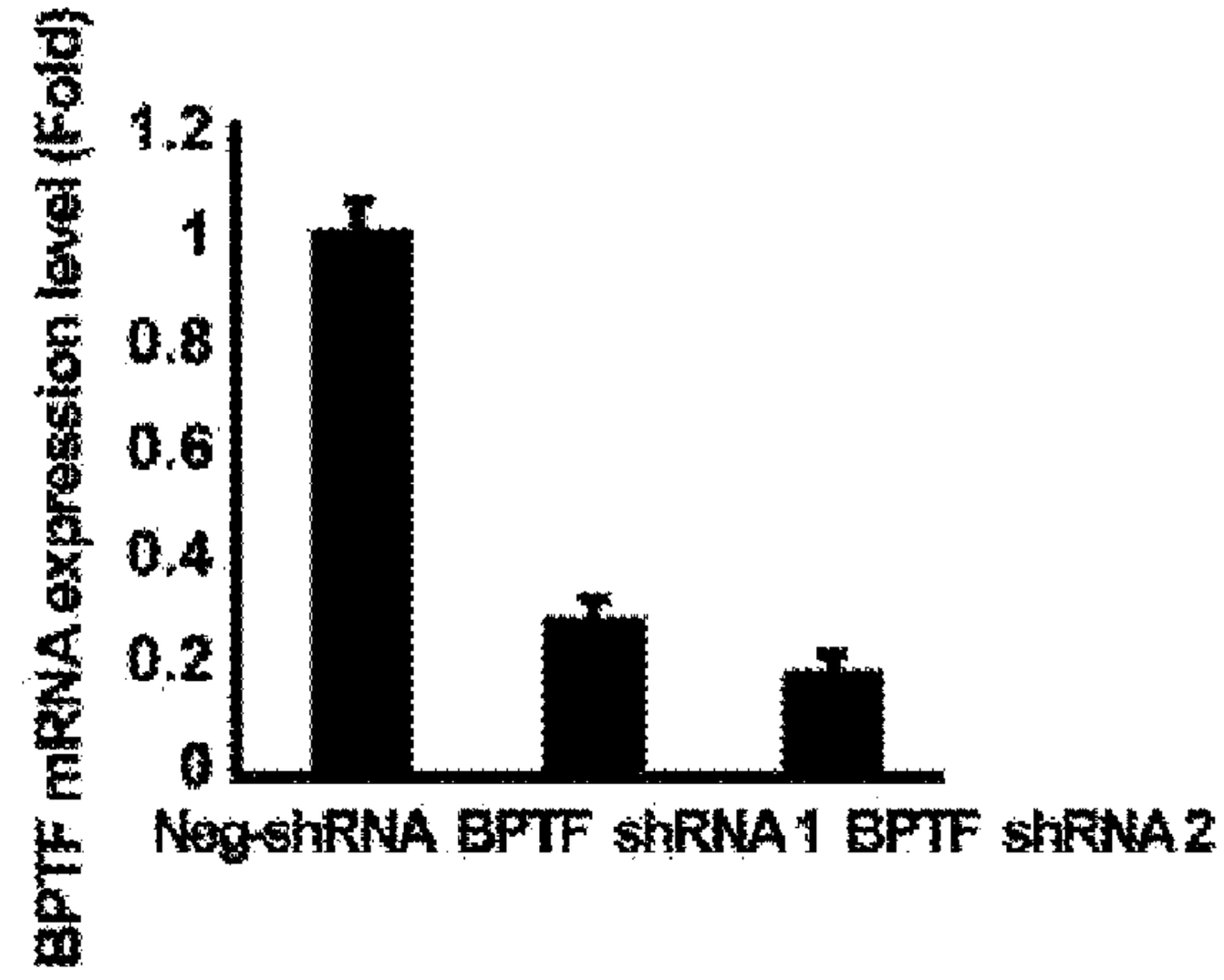

3000
2500
2000
1500
1000
500
0
Volume mm3
MDA-231-BPTF shRNA
MDA-231-Neg shRNA
6D
9D
12D
15D
18D
21D
24D
27D

FIGURE 17

A)


BPTF mRNA expression level (Fold)
1.2
1
0.8
0.6
0.4
0.2
0
Neg-shRNA
BPTF shRNA 1
BPTF shRNA 2