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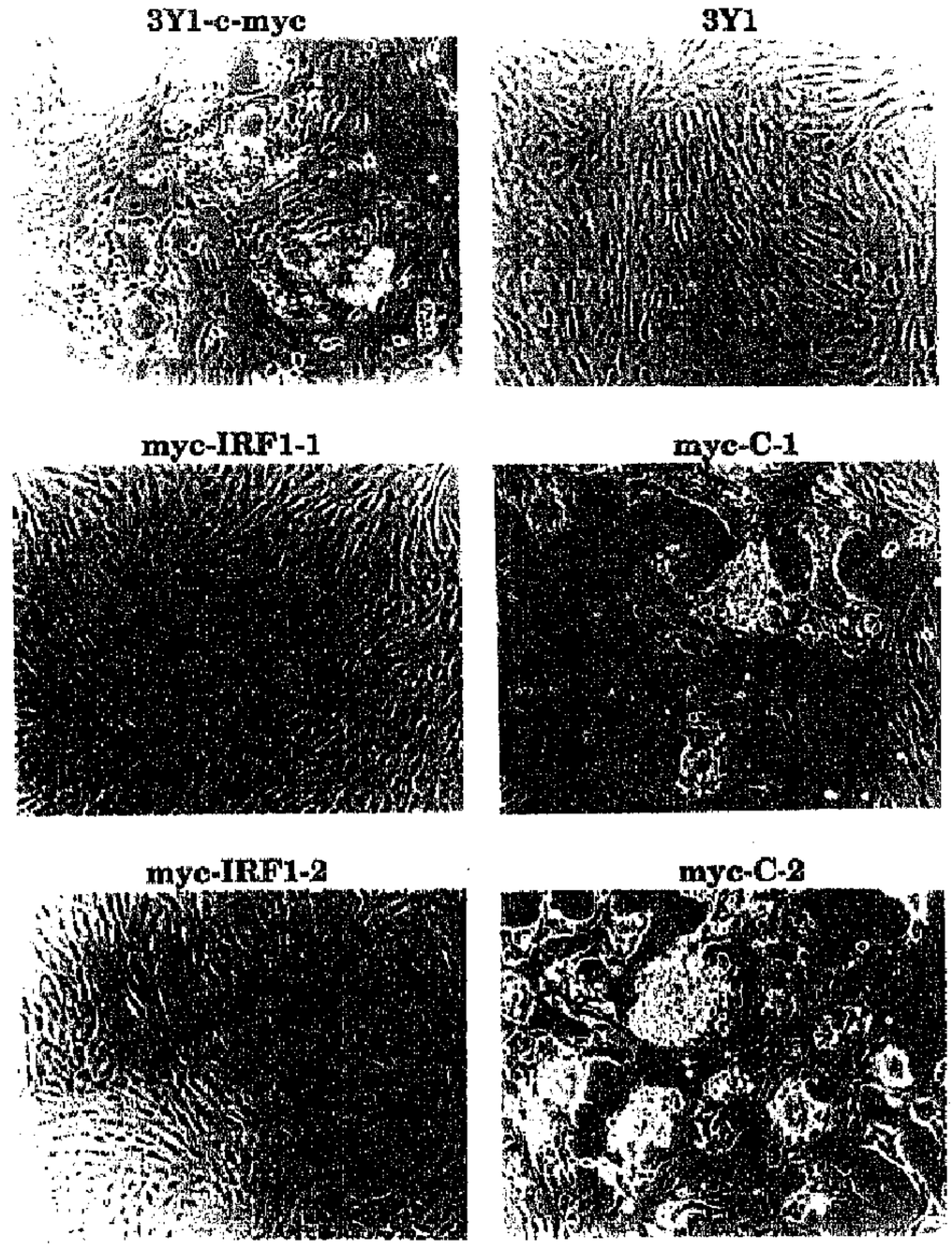
(54) Titre : METHODE D'INVERSION DU PHENOTYPE DE CELLULES TRANSFORMEES PAR LE FACTEUR DE TRANSCRIPTION IRF-I

(54) Title: METHOD TO REVERSE THE PHENOTYPE OF TRANSFORMED CELLS BY THE TRANSCRIPTION FACTOR IRF-1



(57) Abrégé/Abstract:
The invention relates to the use of IRF-1 or nucleic acids containing the coding information for IRF-1 for the reversal of the phenotype of transformed cells. Nucleic acids coding for IRF-1 can be used as tumor suppressor genes in the gene therapy of cancer.

Abstract

The invention relates to the use of IRF-1 or nucleic acids containing the coding information for IRF-1 for the reversal of the phenotype of transformed cells. Nucleic acids coding for IRF-1 can be used as tumor suppressor genes in the gene therapy of cancer.

A method to reverse the phenotype of transformed cells by the transcription factor IRF-1

The present invention relates to the use of the transcription factor interferon regulatory factor-1 (IRF-1) or nucleic acid molecules containing the coding information for IRF-1 to reverse the phenotype of transformed cells.

Interferon regulatory factor-1 (IRF-1), a transcriptional activator, and IRF-2, its antagonistic repressor, have been identified as regulators of type I interferon (IFN) and IFN-inducible genes. It has been shown previously that IRF-1 manifests anti-oncogenic activity in NIH 3T3 cells which are transformed by overexpression of IRF-2. In fact, when the IRF-2 gene was overexpressed in NIH 3T3 cells, the cells became transformed and displayed enhanced tumorigenicity in nude mice. However, this transformed phenotype was reversed by concomitant overexpression of the IRF-1 gene (Harada *et al.*, *Science* **259**, 971-974, 1993). It has been shown that the human IRF-1 gene maps to chromosome 5q31.1, a region frequently deleted in patients with leukemia or preleukemic myelodysplastic sydromes (Willman *et al.*, *Science* **259**, 968-971, 1993).

In the present invention, it has surprisingly been found that the reversal of the transformed phenotype by IRF-1 is not restricted to cells transformed by its natural antagonist, IRF-2, but can also be achieved in cells transformed by oncogenes. So, this invention makes it possible to revert the transformed phenotype in tumor cells more generally. IRF-1 and nucleic acids coding for IRF-1 can be applied in the therapy of cancer, especially in gene therapy.

Thus, the present invention is related to the use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof for the reversal of the phenotype of transformed cells, especially when said cells express an oncogene. Preferably, said oncogene is *c-myc* or *fosB*. It is further preferred that said cells are characterized by lack or shortage of tumor suppressor gene expression. Advantageously, said nucleic acid is incorporated into an expression vector. This can be, for example, a viral vector, preferably a retroviral vector, e.g. pDG. A preferred embodiment of the present invention is the gene transfer of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof into transformed or cancer cells. As a gene transfer vehicle, an appropriate retrovirus can be used. However, in another preferred embodiment said nucleic acid molecule is complexed with a conjugate, said conjugate consisting of an endosomolytical agent and a DNA binding agent (see below). Preferably, said endosomolytical agent is an inactivated adenovirus and

said DNA-binding agent is polylysine. The present invention is further related to the use of a nucleic acid containing the coding information for IRF-1 or a biologically active variant or fragment thereof for the manufacture of a pharmaceutical composition for the therapy of cancer, especially when said pharmaceutical composition is suited for the gene therapy of cancer. In a further aspect, the present invention is related to a pharmaceutical composition for the therapy of cancer, characterized in that it contains a nucleic acid containing the coding information for IRF-1 or a biologically active variant or fragment thereof. Said nucleic acid may also contain regulatory sequences. In one preferred embodiment, the coding information for IRF-1 is incorporated in an nucleic acid molecule which is complexed with a conjugate, said conjugate consisting of an endosomolytical agent and an DNA binding agent. Preferably, said endosomolytical agent is an inactivated adenovirus or adenoviral particles. Preferably, said DNA binding agent is polylysine. In another aspect, the present invention is related to the use of IRF-1 polypeptide or a biologically active variant or fragment thereof for the manufacture of a pharmaceutical composition for the therapy of cancer and to a pharmaceutical composition containing IRF-1 polypeptide or a biologically active variant or fragment thereof. Furthermore, the present invention is related to a method to reverse the phenotype of transformed cells, characterized in that a nucleic acid is transferred into said cells, said nucleic acid containing the coding information for IRF-1 or a biologically active variant or fragment thereof. The IRF-1 of the present invention comes preferably form the same species as the transforemd or cancer cells which are to be treated. If human cells are to be treated, the IRF-1 is preferably human IRF-1. The experts knows from the art how to produce nucleic acid molecules containing the coding information for IRF-1, for example IRF-1 from mouse (Miyamoto *et al.*, *Cell* **54**, 903-913, 1988) or man (Maruyama *et al.*, *Nucl. Acids Res.* **17**, 3292, 1989; EP 0359998). He further knows how to generate nucleic acid molecules coding for variants or fragments and methods to test whether such variants or fragments still exhibit the biological activity of the parent molecule. Thus, such variants and fragments are also encompassed by the present invention. The skilled expert further knows methods from the art how to integrate the coding information for IRF-1 into nucleic acids capable of expressing the gene, and to introduce such nucleic acid molecules into cells in a way that the IRF-1 gene is expressed in the target cells. More detailed examples how the present invention can be carried out are described below.

One aspect of the invention relates to use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the reversal of the phenotype of cells which express the oncogene *c-myc* or *fosB*, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, as a tumor suppressor in the therapy of cancer, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice, and wherein the cancer comprises cells expressing *c-myc* or *fosB*.

Another aspect of the invention relates to use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the manufacture of a pharmaceutical composition for the therapy of cancer, wherein cancer cells of said cancer express the oncogene *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF 1) or a biologically active variant or fragment thereof, as a tumor suppressor in the manufacture of a pharmaceutical composition for the therapy of cancer, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformer or

fosB-transformed cells in nude mice, and wherein the cancer comprises cells expressing *c-myc* or *fosB*.

Another aspect of the invention relates to a pharmaceutical composition for the therapy of cancer, the composition comprising a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof and a pharmaceutically acceptable diluent or carrier, wherein the cancer comprises cells expressing *c-myc* or *fosB* and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to use of interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the manufacture of a pharmaceutical composition for the therapy of cancer wherein cancer cells of said cancer express the oncogene *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to a pharmaceutical composition for the therapy of cancer, the composition comprising interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof and a pharmaceutically acceptable diluent or carrier, wherein the cancer comprises cells expressing *c-myc* or *fosB* and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to an *in vitro* or *ex-vivo* method to reverse the phenotype of transformed cells which express the oncogene *c-myc* or *fosB*, the method

comprising the step of transferring a nucleic acid into said cells, said nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to a commercial package comprising a pharmaceutically effective amount of a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof together with instructions for use thereof for therapy of cancer, wherein the cancer comprises cell expressing *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

Another aspect of the invention relates to a kit comprising in separate containers (a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof for therapy of cancer, wherein the cancer comprises cells expressing *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice; (b) an endosomolytical agent which is an inactivated adenovirus; and (c) a DNA binding agent which is polylysine.

Another aspect of the invention relates to use of (a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1), or (b) a nucleic acid which hybridizes to the complement of the nucleic acid of (a) under moderately stringent hybridization conditions and which codes for a biologically active fragment or variant of IRF-1, wherein

the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice and wherein the moderately stringent hybridization conditions are defined by prewashing with 5 x SSC, 0.5% SDS, 1mM EDTA (ph 8.0) and overnight hybridization at 50°C in 2 x SSC, to treat cancer in a warm-blooded animal, wherein the cancer comprises cells expressing *c-myc* or *fosB*.

Another aspect of the invention relates to a pharmaceutical composition for the therapy of cancer wherein the cancer comprises cells expressing *c-myc* or *fosB*, the composition comprising: (a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1), or (b) a nucleic acid which hybridizes to the complement of the nucleic acid of (a) under moderately stringent hybridization conditions and which codes for a biologically active fragment or variant of IRF-1, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice, and wherein the moderately stringent hybridization conditions are defined by prewashing with 5 x SSC, 0.5% SDS, 1mM EDTA (ph 8.0) and overnight hybridization at 50°C in 2 x SSC together with a pharmaceutically acceptable active diluent or carrier.

Equivalent proteins may vary by one or more substitutions, deletions, or additions, the net effect of which is to retain biological activity of the protein. Alternatively, DNA analog sequences are equivalent to the specific DNA sequences disclosed herein if: (a) the DNA analog sequence comprises sequences derived from biologically active fragments of the IRF-1 gene; or (b) the DNA analog sequence is capable of hybridization to the complement of the DNA sequences of (a) under high or moderate stringent conditions and encodes a biologically active IRF-1 molecule; or (c) DNA analog

sequence is degenerate as a result of the genetic code to the DNA analog sequences defined in (a) or (b) and which encode a biologically active IRF-1 molecule.

Moderately stringent hybridization conditions, as known to those of skill in the art, refer to conditions described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2 Ed. Vol. 1, pages 1.101-1.104 (Cold Spring Harbor Laboratory Press, 1989). Exemplary conditions of moderate stringency are prewashing with 5 x SSC, 0.5% SDS, 1mM EDTA (ph 8.0) and overnight hybridization at 50°C in 2 x SSC. Exemplary severe or high stringency conditions are overnight hybridization at about 68°C in a 6 x SSC solution, washing at room temperature with 6 x SSC solution, followed by washing at about 68°C in a 0.6 x SSC solution.

The inventor of the present invention attempted to revert the phenotype of rat Rat 1 cells transformed by either *c-myc* gene or *fosB* gene. These two genes are well-known oncogenes and their properties have been well studied (Shiroki et al., Mol. Cell. Biol. 6, 4379-4386, 1986; Nakabeppu et al., Mol. Cell. Biol. 13, 4157-4166, 1993). A retrovirus, pDGIRF1, which expresses murine IRF-1 upon infection, was previously constructed (Harada et al., Science 259, 971-974, 1993). The *c-myc*-transformed 3Y1 cell or *fosB*-

transformed Rat-1A cell, termed RmycY1 and Rat-1A(FosB), respectively, both show anchorage-independent growth and enhanced tumorigenicity in rat or nude mice (Shiroki *et al.*, *Mol. Cell. Biol.* **6**, 4379-4386, 1986; Nakabeppu *et al.*, *Mol. Cell. Biol.* **13**, 4157-4166, 1993). 5 x 10^5 of each cells were infected by the pDGIRF-1 virus at the multiplicity of infection (m.o.i.) of 10, using exactly the method employed by Harada *et al.*, and subsequently limiting diluted to obtain single clones. After 1 or 2 weeks, these cell clones have lost the transformed phenotype. In fact, these virally infected cells have changed their morphology which is indistinguishable from the original Rat 1 cells (Figures 1,2), and lost their tumorigenic potential in nude mice (Table 1). In contrast, no such change was observed by infecting similarly the control retrovirus pGD (Figures 1,2). Thus, these observations clearly indicate the broad function of IRF-1 as tumor suppressor in cells transformed by at least two different oncogenes. We also observed that the levels of the oncogene messenger RNAs expressed in these reverted cells remained essentially the same as the original, transformed cells. Therefore, the observed phenotypic reversion of the cells as the result of IRF-1 gene expression is not due to the inhibition of the promoters used for the oncogene expression. The results suggest that some of the cancer cells may be suppressed either completely, as shown here, or partially for their phenotype. Hence, the present invention may be very important for the future development of IRF-1 gene as promising tumor suppressor gene for gene therapy of cancer, especially for cancers which contain activated oncogenes or are characterized by lack or shortage of tumor suppressor gene expression. It can be also emphasized that the IRF-1 gene transfer can be achieved not only by the presently employed retrovirus but also by other means, i.e. the present invention is not restricted to the use of the IRF-1-expressing retrovirus, rather, it points to the general importance of IRF-1 gene transfer in the suppression of tumor cells. Reviews of gene transfer systems and clinical protocols which can be adapted for use of the present invention can be found in the literature (Morgan *et* Anderson, *Ann. Rev. Biochem.* **62**, 191-217, 1993; Roemer *et* Friedmann, *Eur. J. Biochem.* **208**, 211-225, 1992).

A gene transfer system which can be used advantageously in connection with the present invention has been developed recently (Curiel *et al.*, *Proc. Natl. Acad. Sci. USA* **88**, 8850-8854, 1991; Curiel *et al.*, *Am. J. Respir. Cell Mol. Biol.* **6**, 247-252, 1992a; Zatloukal *et al.*, *Ann. New York Acad. Sci.* **660**, 136-153, 1992; Cotten *et al.*, *Proc. Natl. Acad. Sci. USA* **89**, 6094-6098, 1992; Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* **89**, 6099-6103, 1992; Curiel *et al.*, *Human Gene Therapy* **3**, 147-154, 1992b). It is based on the property of adenoviruses to discharge the content of endosomes after having been incorporated by receptor-mediated endocytosis. The use of adenoviruses enhances the efficiency of gene transfer because it circumvents the lysosomal degradation of internalised DNA. The adenovirus can be modified by conjugation with polylysine. Since polylysine is cationic under

physiological conditions, the resulting conjugate can complex DNA, for example DNA coding for IRF-1, by electrostatic interactions. The adenovirus-polylysine conjugates can be used to complex DNA together with transferrin-polylysine conjugates, resulting in ternary adenovirus-polylysine/transferrin-polylysine/DNA complexes (Wagner *et al., loc. cit.*, 1992). Such complexes can bind to receptors specific for adenoviruses and/or transferrin and are internalized afterwards. After endocytosis, the adenovirus causes the break of the endosomal membrane and its content is discharged into the cytoplasm. The IRF-1-coding DNA can enter the cell nucleus, where IRF-1 is expressed mainly by episomally localized DNA.

Table 1: Growth properties of RmycY1 and Rat-1A(FosB) cells overexpressing IRF-1

Cells	*Growth efficiency (%) in methylcellulose gel**	*Tumorigenicity†*	
		Tumors per injection	*Latency (weeks)*
RmycY1	28, 26	6/6	3 to 4
3Y1	0, 0	0/6	
myc-IRF1-1	<1, <1	0/2	
myc-IRF1-2	<1, <1	0/2	
myc-IRF1-3	<1, <1	0/2	
myc-IRF1-4	<1, <1	0/2	
myc-C-1	32, 34	2/2	3 to 4
myc-C-2	30, 28	2/2	3 to 4
Rat-1A(FosB)	24, 28	4/4	2 to 4
Rat-1A	0, 0	0/4	
Fos B-IRF1-1	<1, <1	0/2	
Fos B-IRF1-2	<1, <1	0/2	
Fos B-IRF1-3	<1, <1	0/2	
Fos B-IRF1-4	<1, <1	0/2	
Fos B-C-1	26, 25	2/2	3 to 4
Fos B-C-2	18, 22	2/2	3 to 4

*Cells (5×10^5) were mixed with 1.3% methylcellulose gel dissolved in culture medium and layered onto an agarose bed composed of 0.53% agarose and culture medium (Harada *et al.*, *Science* 259, 971-974, 1993). †Four- to six-week-old nude mice (BALB/c nu/nu) were injected subcutaneously on both franks with 2×10^6 cells and tumors were scored as described by Harada *et al.*.

Brief description of the drawings

Figure 1: Phenotypically reverted c-*myc*-transformed 3Y1 cells following retroviral introduction of *IRF-1*. The cells were all photographed (magnification, x100) in Dulbecco modified Eagle medium containing 10% fetal calf serum. 3Y1-c-myc; c-myc-transformed 3Y1 cells (RmycY1), 3Y1; untransformed rat 3Y1 cells, myc-IRF1-1 and myc-IRF1-2; pGDIRF1 infected RmycY1 cell clones, myc-C-1 and myc-C-2; control virus pGD (Harada *et al.*, *Science* **259**, 971-974, 1993) infected RmycY1 cell clones.

Figure 2: Phenotypically reverted *fosB* transformed Rat-1A cells following retroviral introduction of *IRF-1*. The cells were all photographed (magnification, x100) in Dulbecco modified Eagle medium containing 10% fetal calf serum. Rat-1A-FosB; *fosB* transformed Rat-1A cells (Rat-1A(FosB)), Rat-1A; untransformed Rat-1A cells, Fos B-IRF1-1 and Fos B-IRF1-2; pGDIRF1 infected Rat-1A(FosB) cell clones, Fos B-C-1 and Fos B-C-2; pGD infected Rat-1A(FosB) cell clones.

Example

A retrovirus, pDGIRF1, which expresses murine IRF-1 upon infection, was constructed as previously described (Harada *et al.*, *Science* **259**, 971-974, 1993). For this purpose, mouse IRF-1 cDNA (Miyamoto *et al.*, *Cell* **54**, 903-913, 1988) was inserted into the pGD vector (Daley *et al.*, *Science* **247**, 824, 1990). The DNA constructs were transfected into ψ2 cells (Mann *et al.*, *Cell* **33**, 153, 1983), which subsequently released into the culture medium a high titer (~ 10^6 colony-forming units per milliliter) of virus, as assayed by the ability to confer *neo* resistance to NIH 3T3 cells. Expression of the IRF-1 protein was confirmed by gel-shift analysis. The *c-myc*-transformed 3Y1 cell or *fosB*-transformed Rat-1A cell, termed RmycY1 and Rat-1A(FosB), respectively, both show anchorage-independent growth and enhanced tumorigenicity in rat or nude mice (Shiroki *et al.*, *Mol. Cell. Biol.* **6**, 4379-4386, 1986; Nakabeppu *et al.*, *Mol. Cell. Biol.* **13**, 4157-4166, 1993). 5 x 10^5 of each cells were infected by the pDGIRF-1 virus at the multiplicity of infection (m.o.i.) of 10, using exactly the method employed by Harada *et al.*, and subsequently limiting diluted to obtain single clones. Morphologic investigations and tumorigenicity tests (nude mice model) were performed by standard procedures. After 1 or 2 weeks, these cell clones have lost the transformed phenotype.

CLAIMS:

1. Use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the reversal of the phenotype of cells which express the oncogene *c-myc* or *fosB*, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

2. Use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, as a tumor suppressor in the therapy of cancer, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice, and wherein the cancer comprises cells expressing *c-myc* or *fosB*.

3. The use of claim 1 or 2, wherein said nucleic acid is incorporated into a vector.

4. The use of claim 3, wherein said vector is an expression vector.

5. The use of claim 3, wherein said vector is a viral vector.

6. The use of claim 1 or 2, wherein said nucleic acid is incorporated into a gene transfer vehicle.

7. The use of claim 6, wherein said gene transfer vehicle is a retrovirus.

8. The use of claim 7, wherein said retrovirus is pDGIRF-1.

9. The use of claim 1 or 2, wherein said nucleic acid is complexed with a conjugate, said conjugate consisting of an endosomolytical agent which is an inactivated adenovirus and a DNA-binding agent which is polylysine.

10. Use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the manufacture of a pharmaceutical composition for the therapy of cancer, wherein cancer cells of said cancer express the oncogene *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

11. Use of a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, as a tumor suppressor in the manufacture of a pharmaceutical composition for the therapy of cancer, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice, and wherein the cancer comprises cells expressing *c-myc* or *fosB*.

12. The use of claim 10 or 11, wherein said nucleic acid is incorporated into a gene transfer vehicle.

13. The use of claim 12, wherein said gene transfer vehicle is a virus.

14. The use of claim 13, wherein said virus is a retrovirus.

15. The use of claim 14, wherein said retrovirus is pDGIRF-1.

16. The use of claim 10 or 11, wherein said nucleic acid is complexed with a conjugate, said conjugate consisting of an endosomolytical agent which is an inactivated adenovirus and a DNA-binding agent which is polylysine.

17. A pharmaceutical composition for the therapy of cancer, the composition comprising a nucleic acid containing the coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof and a pharmaceutically acceptable diluent or carrier, wherein the cancer comprises cells expressing *c-myc* or *fosB* and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

18. Use of interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, for the manufacture of a pharmaceutical composition for the therapy of cancer wherein cancer cells of said cancer express the oncogene *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

19. A pharmaceutical composition for the therapy of cancer, the composition comprising interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof and a pharmaceutically acceptable diluent or carrier, wherein the cancer comprises cells expressing *c-myc* or *fosB* and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

20. An *in vitro* or *ex-vivo* method to reverse the phenotype of transformed cells which express the oncogene *c-myc* or *fosB*, the method comprising the step of transferring a nucleic acid into said cells, said nucleic acid containing the

coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

21. A commercial package comprising a pharmaceutically effective amount of a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof together with instructions for use thereof for therapy of cancer, wherein the cancer comprises cell expressing *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice.

22. A kit comprising in separate containers (a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1) or a biologically active variant or fragment thereof for therapy of cancer, wherein the cancer comprises cells expressing *c-myc* or *fosB*, and wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice; (b) an endosomolytical agent which is an inactivated adenovirus; and (c) a DNA binding agent which is polylysine.

23. Use of

(a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1), or

(b) a nucleic acid which hybridizes to the complement of the nucleic acid of (a) under moderately stringent hybridization conditions and which codes for a biologically active fragment or variant of IRF-1, wherein the

variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice and wherein the moderately stringent hybridization conditions are defined by prewashing with 5 x SSC, 0.5% SDS, 1mM EDTA (pH 8.0) and overnight hybridization at 50°C in 2 x SSC, to treat cancer in a warm-blooded animal, wherein the cancer comprises cells expressing *c-myc* or *fosB*.

24. A pharmaceutical composition for the therapy of cancer wherein the cancer comprises cells expressing *c-myc* or *fosB*, the composition comprising:

(a) a nucleic acid containing coding information for interferon regulatory factor 1 (IRF-1), or

(b) a nucleic acid which hybridizes to the complement of the nucleic acid of (a) under moderately stringent hybridization conditions and which codes for a biologically active fragment or variant of IRF-1, wherein the variant or fragment reduces anchorage-independent growth and reduces tumorigenicity of *c-myc*-transformed or *fosB*-transformed cells in nude mice, and wherein the moderately stringent hybridization conditions are defined by prewashing with 5 x SSC, 0.5% SDS, 1mM EDTA (pH 8.0) and overnight hybridization at 50°C in 2 x SSC,

together with a pharmaceutically acceptable active diluent or carrier.

FETHERSTONHAUGH & CO.

OTTAWA, CANADA

PATENT AGENTS

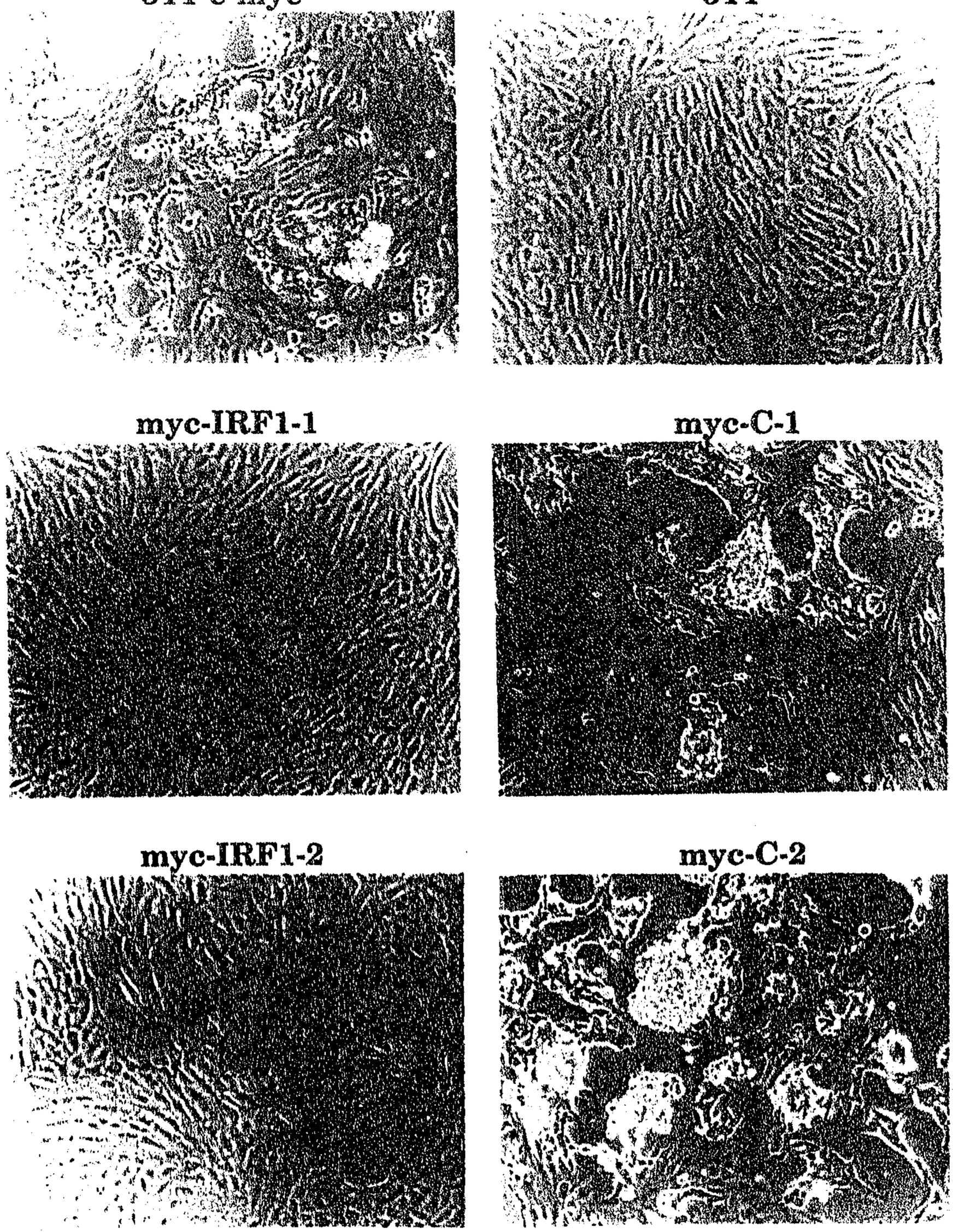


Fig. 1

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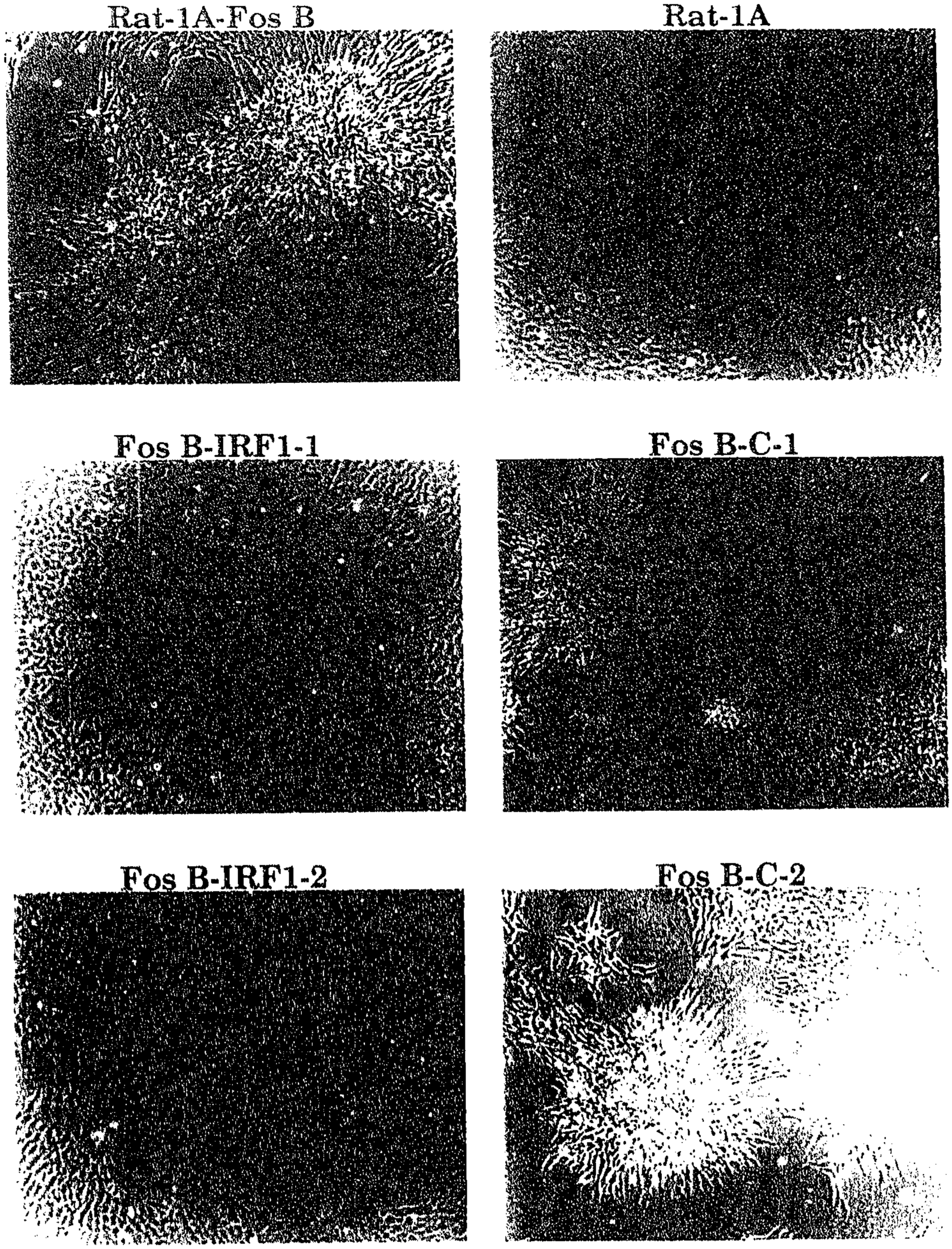
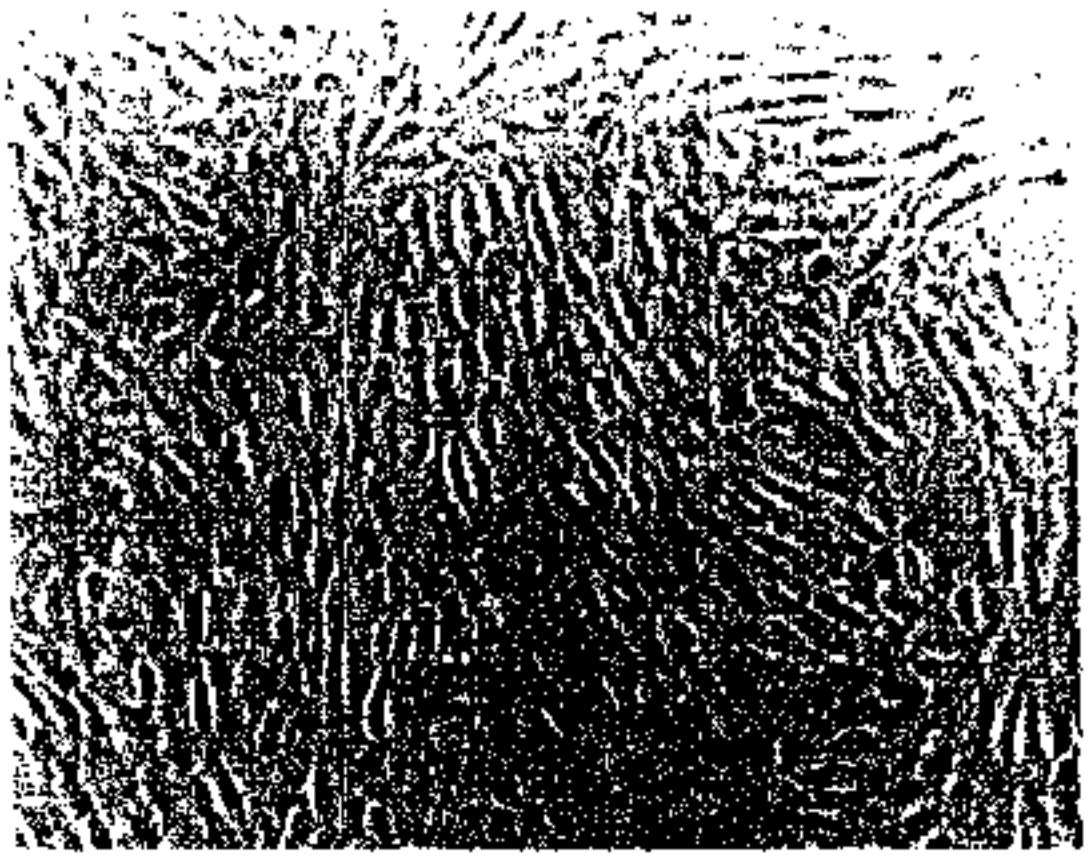


Fig. 2

3Y1-c-myc

3Y1



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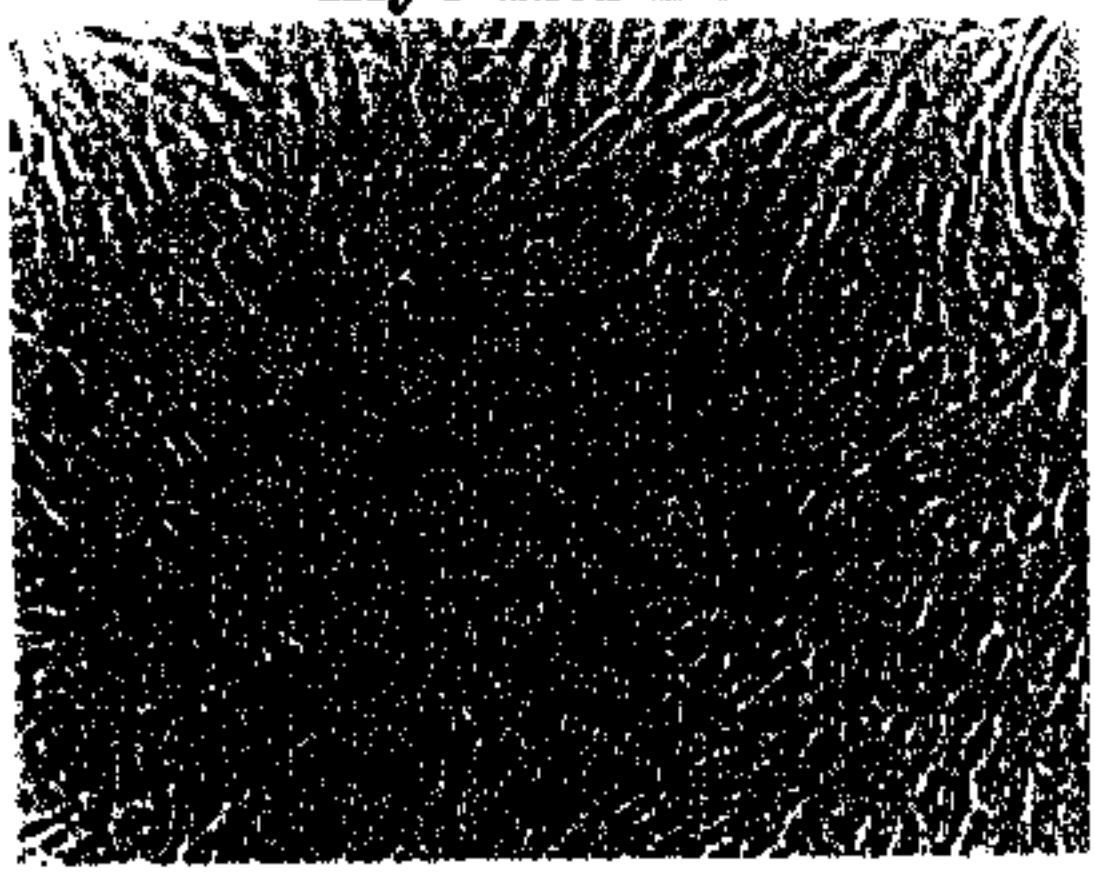

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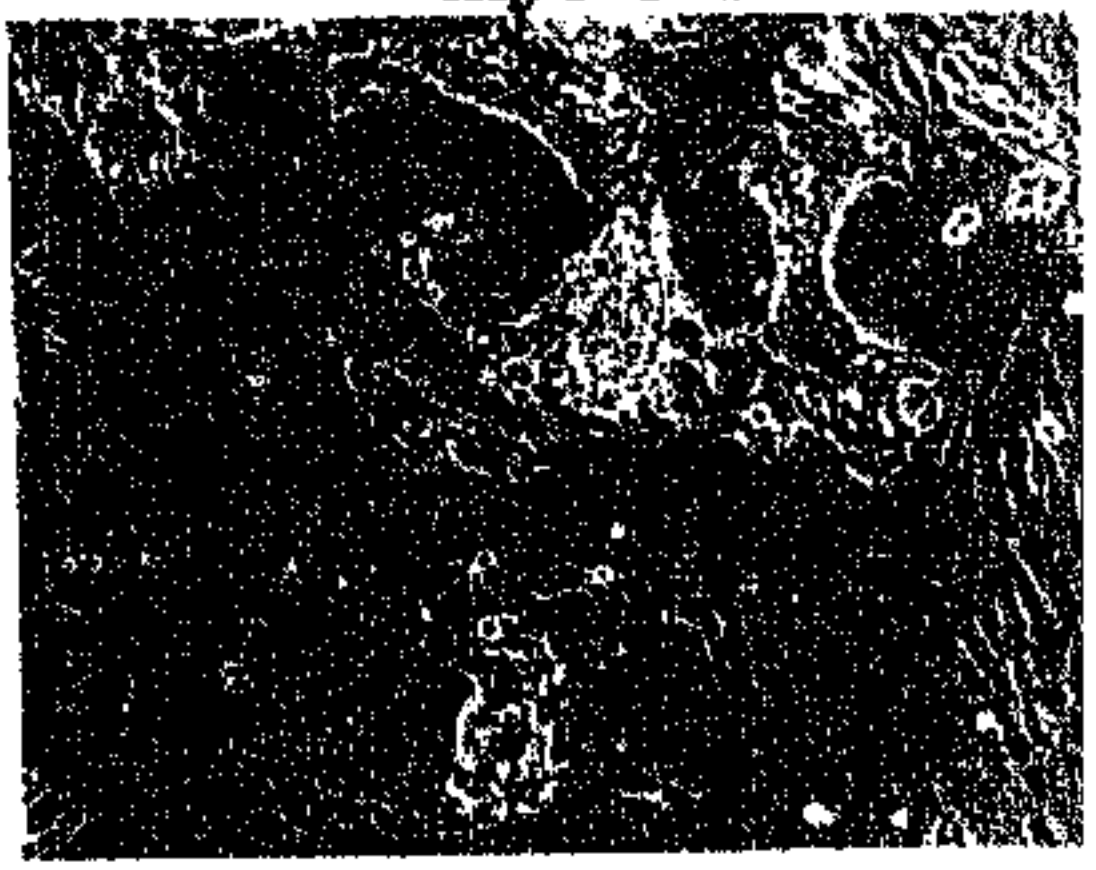

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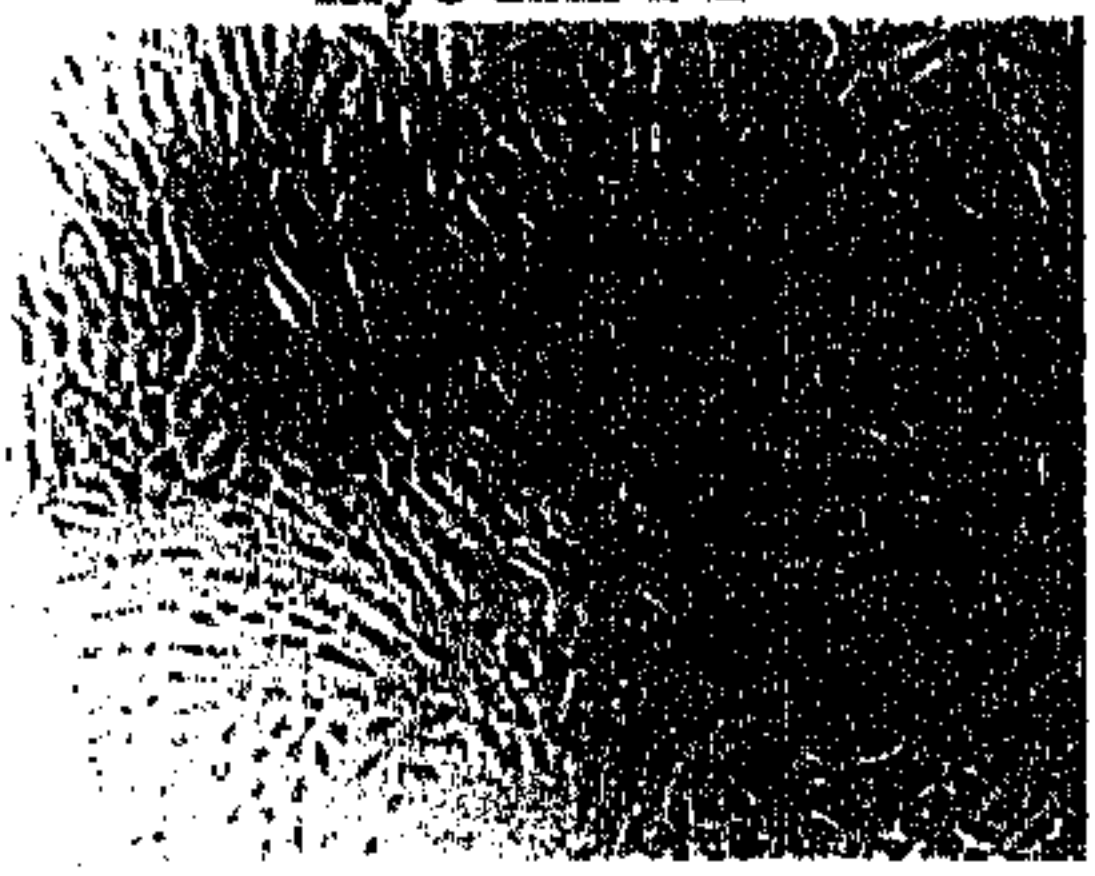

myc-C-2