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(71) Applicants: SOCIETE DE DEVELOPPEMENT ET DE RECHERCHE INDUSTRIELLE [FR/FR]; 42 Rue de Longvic, F-21300 Chenove (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE - CNRS - [FR/FR]; 3, rue Michel Ange, F-75794 Paris Cedex 16 (FR). INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE) [FR/FR]; 101, rue de Tolbiac, F-75013 Paris (FR). UNIVERSITÉ PARIS DIDEROT - PARIS 7 [FR/FR]; 5, rue Thomas Mann, F-75013 Paris (FR). ECOLE NORMALE SUPERIEURE [FR/FR]; 45 rue d'Ulm, F-75005 Paris (FR).

(72) Inventors: DUGAST-DARZACQ, Claire; 24 avenue Raymond Aron, F-92160 Antony (FR). NOIZET, Maïté; 76-78 Avenue de Paris, Bâtiment Le Vau, F-78000 Versailles (FR). DARZACQ, Xavier; 24 avenue Raymond Aron, F-92160 Antony (FR).

(74) Agents: BERNARDI, Céline et al.; Cabinet Plasseraud, 52 rue de la Victoire, F-75440 Paris Cedex 09 (FR).

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(54) Title: MOLECULAR TARGETS FOR THE PREVENTION AND/OR TREATMENT OF FIBROSIS, HYPERTROPHIC SCARS OR KELOIDS

(57) Abstract: The present invention relates to a therapeutic compound comprising : - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, and/or - an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2 for use in the prevention and/or treatment of fibrosis, hypertrophic scar or keloid.



## **Molecular targets for the prevention and/or treatment of fibrosis, hypertrophic scars or keloids**

### **Field of the invention**

The present invention relates to at least one molecular target for the prevention and/or treatment of fibrosis, hypertrophic scars or keloids. Further, the invention concerns a novel therapeutic for preventing or treating fibrosis, hypertrophic scars or keloids and a novel gene therapy approach, involving said molecular target for preventing and/or treating said fibrosis, hypertrophic scars or keloids. Additionally, the invention concerns a method for preventing and/or treating fibrosis, hypertrophic scars or keloids using said therapeutic or said gene therapy.

### **Background of the invention**

Fibrosis is the formation of excess fibrous tissues or scar tissue in an organ or a tissue. Fibrosis is a common pathophysiological response of tissues to chronic injury or long-term inflammation. There are many potential origins of this fibrosis. It can be induced by a disease (inherited or not), by side effects of a treatment (for example radiation or chemotherapy), by a toxic environment (for example smoking), or by an injury. It can affect different organs such as the skin or the lung.

Fibrosis induced generally the failure of the tissue of the organ that is affected. Fibrotic tissue is like a scar tissue, stiff, thick, and rigid. Sometimes, it can also swell. For example, in the lung, fibrosis lead to a shortness of breath particularly during exercise and dry and hacking cough, due to the abnormal expansion of the fibrosis lung.

Some examples of fibrosis are pulmonary fibrosis (lungs), cystic fibrosis (lung and digestive system), Crohn's Disease (intestine), scleroderma/systemic sclerosis (lungs or skin), arthrofibrosis (knee, shoulder, other joints), cutaneous fibrosis with hypertrophic or keloid scars

When the tissue concerned by this phenomenon is the skin, it is principally the wound healing process that is affected.

The natural wound healing is divided into three sequential phases; each phase is characterized by specific cellular activities: the inflammatory phase, the proliferative phase and the remodeling phase.

5 The first phase, called the inflammatory phase, begins minutes after injury. The blood vessels rupture induces the clot formation, composed mainly of fibrin and fibronectin. The clot fills partially the lesion and allows the migration of the inflammatory cells within the lesion. The inflammatory cells are recruited to debride the wound. Platelets secrete factors, such as growth factors or cytokines, which induce the recruitment of cells implicated in the wound healing (inflammatory cells such as neutrophils and macrophages, fibroblasts and endothelial  
10 cells).

The second phase is called the proliferative phase and corresponds to the development of the granulation tissue. Fibroblasts migrate into the wound area, proliferate and form a new provisional extracellular matrix by secreting extracellular matrix (ECM) proteins. Then endothelial cells migrate to promote the neovascularization or angiogenesis of the lesion.  
15 Inside the granulation tissue, fibroblasts activate and differentiate into myofibroblasts, presenting contractile properties thanks to their expression of alpha-smooth muscle actin (similar to that in smooth muscle cells). Myofibroblasts have a key role in wound healing as they provide the contraction of the wound. Finally, keratinocytes migrate from the wound edge, proliferate and differentiate to reconstitute the epidermis.

20 The last phase of the wound healing process appears after the wound closure. It corresponds to the remodeling of the granulation tissue. The granulation tissue is reorganized, type III collagen is replaced by type I collagen, as normal dermis is principally composed of type I collagen. During this phase, myofibroblasts in excess are eliminated by apoptosis. The last phase of the wound healing is long. One year after injury, the scar is remodeled; it gets less  
25 red and thinner.

However, this process is not only complex but fragile; it is susceptible to interruption or failure leading to the formation of chronic or non-healing wounds or formation of abnormal scars. Factors which may contribute to this include diseases (such as diabetes, venous or arterial disease), age, infection or tissue localization.

30 **Role of fibroblasts in wound healing**

Fibroblasts are implicated in the process of wound healing, this involves several steps of differentiation from a quiescent fibroblast to a mobilized fibroblast that will transform into a myofibroblast and finally enter apoptosis.

In normal wound healing, fibroblasts get activated, and then differentiate into myofibroblasts presenting contractile properties thanks to their expression of alpha-smooth muscle actin ( $\alpha$ SMA). Myofibroblasts are responsible for the deposit of extra cellular matrix and for the wound closure by moving closer the wound edges. In hypertrophic scar, keloid or fibrous wound healing, the activity of myofibroblasts persists and leads to tissue deformation, which is particularly evident, for example, in hypertrophic scars developed after burn injury. The aim of the present invention is to map, at the whole genome scale, the different genes that will be activated or deactivated during this process, and thus providing a molecular signature of abnormal healing leading to abnormal scar or fibrosis

Connective tissues represent a wide variety of physical structures and different functions: tendons, cartilage, bone, dermis, cornea, etc... As organs and tissues have specific functions (for example, skin functions are protection, sensation and heat regulation), connective tissues constituting these tissues and organs have also precise functions provided by specific cell types. For example, in the papillar or reticular dermis collagen I, III and V, XIV, elastic fibers, perlecan or SPARC are found On the contrary, types III, IX, X collagens are found associated with aggregan and dermatan sulfate in tendons.

Fibroblasts are the main cells of connective (or mesenchymal) tissues, in which cells are surrounded by extracellular matrix (contrary to epithelium where they are jointed together). These fibroblasts are active in wound healing of damages organs, as they proliferate, differentiate in myofibroblast, secrete collagens and other specific ECM proteins and fibers composing the connective tissue of the organ, leading to the healing and reorganization of the tissue.

Myofibroblasts are defined as the primary source of the excessive ECM proteins deposition occurring during fibrosis. Resident myofibroblasts arise from a population of tissue specific fibroblasts that proliferate and undergo activation in response to injury, as it is the case in many organs such as skin, lungs, or kidney.

30

### **Fibrosis, hypertrophic scars and keloids**

Fibrosis is a common pathophysiological response of tissues to chronic injury. Fibrosis affects different organs such as the skin or the lung. Fibrosis is characterized by a differentiation of fibroblasts into myofibroblasts and an excessive accumulation of connective tissue. Fibrosis induces a loss of function of the organ and potentially the failure of the organ.

- 5 Hypertrophic, keloid or fibrous scars result from abnormal wound healing. These scars are characterized by an excessive deposit of ECM proteins, especially collagen. In these abnormal wounds, granulation tissue is hyper proliferative, due to an excess of myofibroblasts (« Cellular and molecular pathology of HTS: basis for treatment. » Armour A, Scott PG, Tredget EE. *Wound Repair Regen.* 2007 Sep-Oct; 15 Suppl 1:S6-17. Review. Erratum in:  
10 *Wound Repair Regen.* 2008 Jul-Aug;16(4):582).

In normal wound healing, fibroblasts get activated, and then differentiate into myofibroblasts presenting contractile properties thanks to their expression of alpha-smooth muscle actin ( $\alpha$ SMA). Myofibroblasts are responsible for the deposit of extra cellular matrix and for the wound closure by moving closer the wound edges. In hypertrophic scar, keloid or fibrous  
15 wound healing, the activity of myofibroblasts persists and leads to tissue deformation, which is particularly evident, for example, in hypertrophic scars developed after burn injury.

Hypertrophic and keloid scars are characterized by deposit of excessive amounts of collagen leading to a raised scar (more intense in keloids than in hypertrophic scars). They are formed most often at the sites of pimples, body piercings, cuts and burns.

- 20 Some hypertrophic scars are non-functional scars as they limit the function of the skin where they developed. They generate a loss of mobility of the scar zone and the neighboring zones, which can completely limit the movements (for example, elbow and mobility of the arm). They are mostly the result of burns of specific anatomical zones.

Thus, the treatment of the wound is especially adapted to the wound in its early stage if it  
25 presents a risk of developing an abnormal scar or failing to heal correctly. By enhancing or manipulating factors that contribute to wound healing it may therefore be possible to correct the process, thereby reducing the likely occurrence of a fibrosis, hypertrophic scar or keloid. If a tissue (of an organ for example) is susceptible of developing a fibrosis, the treatment of this tissue at an early stage is also of interest.

- 30 The present invention can improve the quality of a patient's life by ensuring that new wounds do not deteriorate into a fibrosis, hypertrophic scar or keloid and existing wounds can be

treated in a way that actively promotes healing and by the way prevents the formation of fibrosis, hypertrophic scar or keloid.

## SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a therapeutic compound comprising:

- 5           - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4

and/or

- 10           - an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2  
for use in the prevention and/or treatment of fibrosis, hypertrophic scar or keloid.

The present invention also relates to a pharmaceutical composition comprising a therapeutic compound as defined above together with a pharmaceutically acceptable carrier.

- 15           The invention also relates to a method for preparing a pharmaceutical composition as described above comprising bringing said therapeutic compound in conjunction or association with a pharmaceutically or veterinary acceptable carrier or vehicle.

In one aspect, the invention relates to a method for treating or preventing mammalian fibrosis, hypertrophic scar or keloid wherein said method comprises administering to said fibrosis,  
20           hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid a therapeutic compound comprising:

- an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4

25           and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2.

In another aspect, the invention relates to a kit for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid wherein said kit  
30           comprises:

(a) at least one therapeutic compound or composition as defined above and

(b) at least one dressing for applying to said wound.

The invention also relates to a combination therapeutic for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid comprising:

(a) - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4

and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

and

b) at least one further therapeutic.

In yet another aspect, the invention relates to the use of:

- an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4

and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

for treating fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid

wherein said agent modulates fibroblast and myofibroblast differentiation and/or activity

**DETAILED DESCRIPTION OF THE INVENTION**

Accordingly, in one aspect of the invention there is provided a therapeutic compound comprising:

(a) an agent that inhibits the activity of at least one gene selected from the group consisting of:

FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4 preferentially TCF4, FOXS1, STAT4

5 an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

for use in the prevention and/or treatment of fibrosis, hypertrophic scars or keloids.

In a preferred embodiment of the invention, said therapeutic compound also comprises:

10 an agent that inhibits the activity of at least one gene selected from the group consisting of E2F1, EGR2, GLI1, JUN, MYC, SMAD3, SMAD4, SOX9, SRF, preferentially EGR2, SOX9

an agent that enhances the activity of at least one gene selected from the group consisting of ETS1, PPARG

for use in the prevention and/or treatment of fibrosis, hypertrophic scars or keloids.

15 Indeed, the inventors have found that these genes were involved in the fibrosis process and that their down-regulation or up-regulation would be useful for the treatment of fibrosis, hypertrophic scars or keloids.

Thus, the invention involves a novel gene therapy approach and/or a novel protein therapy approach.

20 As used herein, the expression “agent that inhibits the activity of a gene” or “inhibitor” refers to an agent than can downregulate said gene. It encompasses agents that act at the level of the gene expression, as well as agents that act at the level of the protein, either by decreasing the amount of protein present in a given cell, or by inhibiting the protein’s activity.

25 As used herein, the expression “agent that enhances the activity of a gene” or “enhancer” refers to an agent than can upregulate said gene. It encompasses agents that act at the level of the gene expression, as well as agents that act at the level of the protein, either by increasing the amount of protein present in a given cell, or by increasing the protein’s activity. It also encompasses agents that act upstream or downstream of said gene or protein in a signaling pathway.

In one embodiment the novel therapeutic comprises an inhibitor or enhancer of the gene expression, this inhibitor or enhancer can be either an anti-sense DNA or RNA, siRNA, shRNA, cDNA, TALENS or ribozymes, either naked or in the form of plasmid and viral vectors or a drug.

In a preferred embodiment, said therapeutic compound is a siRNA selected from the group consisting of siRNA having the sequence as set forth in SEQ ID No:11 to SEQ ID No:74 and mixtures thereof:

Target gene	Sequence (5' --> 3')	SEQ ID No:
<i>CREB5</i>	CCGGCGAAGGGUGGUAGACGA	11
	AACAGUAUUCUGUAGGAUCUA	12
<i>E2F1</i>	UCGGAGAACUUUCAGAUCU	13
	GAGAAGUCACGCUAUGAGA	14
	GAGCAGAUGGUUAUGGUGA	15
	GAACAGGGCCACUGACUCU	16
<i>EGR2</i>	GAAGGCAUAAUCAUAUUG	17
	CUACUGUGGCCGAAAGUUU	18
	GAAACCAGACCUUCACUUA	19
	GAGAAGAGGUCGUUGGAUC	20
<i>FOXS1</i>	AGGGCCAAUAAAGCCAUGUGA	21
	CUGGCUCUAGGACCUGAAGAA	22
<i>GLI1</i>	GCAAAUAGGGCUUCACAUUA	23
	AGGCUCAGCUUGUGUGUAA	24
	GGACGAGGGACCUUGCAUU	25
	CAGCUAGAGUCCAGAGGUU	26
<i>HIC1</i>	GCACAGCAACGCAACCUCA	27
	GAGCUUCGGUGACAACCUG	28
	UGAUCAUCGUGGUGCAGAA	29

	GACCAUCGACCGUUUCUCU	30
<i>IRF7</i>	GCACAAGGUGUACGCGCUC	31
	CAACUUCCGCUGCGCACUG	32
	GCGCGCAUCUUCAAGGCCU	33
	CAGGCACGGACCAGACUGA	34
<i>JUN</i>	GAGCGGACCUUAUGGCUAC	35
	GAACAGGUGGCACAGCUUA	36
	GAAACGACCUUCUAUGACG	37
	UGAAAGCUCAGAACUCGGA	38
<i>MYC</i>	ACGGAACUCUUGUGCGUAA	39
	GAACACACAACGUCUUGGA	40
	AACGUUAGCUUCACCAACA	41
	CGAUGUUGUUUCUGUGGAA	42
<i>SMAD3</i>	CAACAGGAAUGCAGCAGUG	43
	GAGUUCGCCUUCAAUAUGA	44
	GGACGCAGGUUCUCCAAC	45
	UUAGAGACAUCAAGUAUGG	46
<i>SMAD4</i>	GCAAUUGAAAGUUUGGUA	47
	CCCACAACCUUAGACUGA	48
	GAAUCCAUAUCACUACGAA	49
	GUACAGAGUUACUACUUAG	50
<i>SOX9</i>	GGAACAACCCGUCUACACA	51
	GAACAAGCCGCACGUCAAG	52
	GACCUUCGAUGUCAACGAG	53
	GGAAGUCGGUGAAGAACGG	54
<i>SRF</i>	UGAGACAGGCCAUGUGUAU	55
	GGACUGUGCUGAAGAGUAC	56
	GCACCAAGAGUGAAUGAUC	57

	GCACCAGUGUCUGCUAGUG	58
<i>STAT1</i>	GCACGAUGGGCUCAGCUUU	59
	CUACGAACAUGACCCUAUC	60
	GAACCUGACUCCAUGCGG	61
	AGAAAGAGCUUGACAGUAA	62
<i>STAT4</i>	GAACUAAACUAUCAGGUA	63
	GCAUGUAGCUGUGGUUAUU	64
	CAAUCUAGCUACAGUGAUG	65
	CUGCGAGACUACAAAGUUA	66
<i>TCF4</i>	GCACUUGCUUCGAUCUAUU	67
	GACAAAGAGCUGAGUGAUU	68
	GCACAGCUGUUUGGUCUAG	69
	CAACGGGACAGACAGUAUA	70
<i>USF2</i>	GCAAGACGGGAGCGAGUAA	71
	GGAGGGACAAGAUAACAA	72
	GAAGAGCCCAGCACAAACGA	73
	CAAAAUCCCUUCAGCAAUG	74

These siRNA inhibit the expression of the target genes.

In one embodiment, the agent that enhances the activity of a gene is a cDNA.

Typically, the expression of the CREB5 gene can be enhanced by administering the CREB5 cDNA.

- 5 The same applies mutatis mutandis for all the other genes listed.

The cDNA can be administered or delivered to the fibrosis, hypertrophic scar or keloid to be treated in any suitable form known to the skilled person in art. It can be delivered as naked DNA, using plasmid vectors, viral vectors, or any other suitable means.

- 10 In another embodiment, the novel therapeutic comprises an inhibitor or enhancer of the protein encoded by the gene function, this inhibitor or enhancer can be either a binding agent

that binds, either reversibly or irreversibly, to inhibit or enhance protein function such as an antibody or a known, or synthesized, protein agonist or antagonist; or an agent that works upstream or downstream of the protein signaling mechanism to inhibit or enhance the protein signaling and so negate or enhance the effects of expression of the protein in wound tissue.

- 5 Said agent (inhibitor or enhancer) can be any agent known in the art to act upon a given molecular target.

Typically, the enhancer of PPARG can be selected in the group consisting of thiazolidinediones, such as rosiglitazone and pioglitazone (Curr Drug Targets Cardiovasc Haematol Disord. 2005 Oct;5(5):377-86. Role of PPAR- gamma agonist thiazolidinediones in  
10 treatment of pre-diabetic and diabetic individuals: a cardiovascular perspective. Dumasia R, Eagle KA, Kline-Rogers E, May N, Cho L, Mukherjee D).

Typically, the inhibitor of PPARG can be G3335 (CAS 36099-95-3) (Chembiochem. 2006 Jan;7(1):74-82. The dipeptide H-Trp-Glu-OH shows highly antagonistic activity against PPARgamma: bioassay with molecular modeling simulation. Ye F, Zhang ZS, Luo HB, Shen  
15 JH, Chen KX, Shen X, Jiang HL.).

The therapeutic of the invention is for use in the treatment or prevention of fibrosis, hypertrophic scar or keloid. These fibrosis, hypertrophic scars or keloids are preferentially mammalian fibrosis, hypertrophic scars or keloids, more preferentially human fibrosis,  
20 hypertrophic scars or keloids.

An antibody for use in the invention is most ideally a monoclonal antibody or a humanized antibody.

In the above aspects and embodiments of the invention the therapeutic is formulated for topical application, but it can also be formulated for oral, cutaneous, transcutaneous,  
25 transdermal, intravenous or any application known.

Alternatively, in the above aspects of the invention the therapeutic is formulated for application into a dressing or impregnation of a dressing.

The therapeutic of the invention may be administered with an active agent. Such active agent may be an antibiotic or antibacterial agent, an antiseptic, an antiviral, an antifungal, an

analgesic, an anti-inflammatory agent, a wound healing agent, a keratolytic agent, an anesthetic agent. Such actives are well known to skilled practitioners.

In another aspect of the invention, there is provided a pharmaceutical composition for use in treating fibrosis, hypertrophic scar or keloid comprising a therapeutic of the invention  
5 together with a pharmaceutical acceptable carrier.

Other active materials may also be present in the pharmaceutical composition, as may be considered appropriate or advisable for the fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid being treated.

The carrier, or, if more than one be present, each of the carriers, must be acceptable in the  
10 sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

The formulations include those suitable for topical, oral, rectal, nasal or any administration known and may be prepared by any methods well known in the art of pharmacy.

The composition may be prepared by bringing into association the therapeutic of the  
15 invention and the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely bringing into association the active with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. The invention extends to methods for preparing a pharmaceutical composition comprising bringing a therapeutic of the invention in conjugation  
20 or association with a pharmaceutically acceptable carrier or vehicle.

For topical application to the skin, compounds of conventional use may be made up into a cream, ointment, gel, jelly, solution or suspension etc. Cream or ointment formulations that may be used for the composition are conventional formulations well known in the art.

Formulations for oral administration in the present invention may be presented as: capsules,  
25 sachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion; or as a bolus etc.

For compositions for oral administration (e.g. tablets and capsules), the term "acceptable  
30 carrier" includes vehicles such as common excipients e.g. binding agents, for example syrup,

acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and  
 5 lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring and the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

10 Additionally, or alternatively, the further aspect of the invention also, or alternatively, comprises a novel method for preventing and/or treating fibrosis, hypertrophic scar or keloid which method comprises:

administering to said fibrosis, hypertrophic scar or keloid:

15 an agent that inhibits the activity of at least one gene selected in the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4 preferentially TCF4, FOXS1, STAT4

and/or

-an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2 and SIX2.

20 The invention also comprises a novel method for preventing and/or treating fibrosis, hypertrophic scar or keloid which method further comprises administering to said fibrosis, hypertrophic scar or keloid:

-an agent that inhibits the activity of at least one gene selected from E2F1, EGR2, GLI1, JUN, MYC, SMAD3, SMAD4, SOX9 and SRF preferentially EGR2, SOX9

25 and/or

- an agent that enhances the activity of at least one gene selected from ETS1, PPARG.

According to yet a further aspect of the invention there is provided a kit for preventing and/or treating fibrosis, hypertrophic scars or keloids which method comprises, wherein said kit comprises:

(a) at least one therapeutic as above described; and

(b) at least one medical device for applying to said fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid.

The term "medical device" includes an instrument, apparatus, implant, in vitro reagent, or similar or related article that is used to diagnose, prevent, or treat disease or other conditions, and does not achieve its purposes through chemical action within or on the body (which would make it a drug). Whereas medicinal products (also called pharmaceuticals) achieve their principal action by pharmacological, metabolic or immunological means, medical devices act by other means like physical, mechanical, or thermal means.

10 According to a yet further aspect of the invention there is provided a combination therapeutic for preventing and/or treating fibrosis, hypertrophic scars or keloids comprising an inhibitor or enhancer of gene expression and an inhibitor or enhancer of protein activity.

According to a further aspect of the invention there is provided a therapeutic for preventing and/or treating fibrosis, hypertrophic scars or keloids an inhibitor or enhancer of protein, or a  
15 homologue thereof.

According to a further aspect of the invention there is provided use of an inhibitor or enhancer of protein, or a homologue thereof, in the manufacture of a medicament for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid. According to a further aspect of the invention there is provided use of an inhibitor or  
20 enhance of protein, or a homologue thereof, for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid. The term "homologue" as used herein refers to amino acid sequences which have a sequence at least 50% homologous to the amino acid sequence of FOXS1, CREB5, E2F1, EGR2, ETS1, GLI1, HIC1, IRF7, JUN, MAF, MEOX2, MYC, POU2F2, PPARG, SIX2, SMAD3, SMAD4,  
25 SOX9, SRF, STAT4, TCF4 and which retain the biological activity of the FOXS1, CREB5, E2F1, EGR2, ETS1, GLI1, HIC1, IRF7, JUN, MAF, MEOX2, MYC, POU2F2, PPARG, SIX2, SMAD3, SMAD4, SOX9, SRF, STAT4, TCF4 sequence. It is preferred that homologues are at least 75% homologous to the FOXS1, CREB5, E2F1, EGR2, ETS1, GLI1, HIC1, IRF7, JUN, MAF, MEOX2, MYC, POU2F2, PPARG, SIX2, SMAD3, SMAD4,  
30 SOX9, SRF, STAT4, TCF4 peptide sequence and, in increasing order of preference, at least 80%, 85%, 90%, 95% or 99% homologous to the FOXS1, CREB5, E2F1, EGR2, ETS1,

GLI1, HIC1, IRF7, JUN, MAF, MEOX2, MYC, POU2F2, PPARG, SIX2, SMAD3, SMAD4, SOX9, SRF, STAT4, TCF4 peptide sequence.

Treatment of fibrosis, hypertrophic scar or keloid described herein includes reference to human or veterinary use.

- 5 In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprises", or variations such as "comprises" or "comprising" is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.
- 10 Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking, the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including the accompanying claims and drawings).

- 15 Thus, features, integers, characteristics, compounds or chemical moieties described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein, unless incompatible therewith.

- Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an  
20 alternative feature serving the same or a similar purpose.

The full identity of the genes according to the invention is available on the NCBI database (<http://www.ncbi.nlm.nih.gov/>), or is well known to those skilled in the art.

The present invention will now be described by way of the following examples with particular reference to Figures and Table wherein:

25 **FIGURE LEGENDS**

**Figure 1:** Key transcription factors in fibroblast to myofibroblast differentiation

a) Graphical explanation of the in silico gene network analysis

b) Table representing the different transcription factors identified by bioinformatical and network analysis.

**Figure 2a:** Graphic representation of the  $\alpha$ SMA mRNA levels as assessed by RT-qPCR. NHDFs were treated either with mock siRNA or siRNA directed against different mRNA (FOXS1, EGR2, SRF, SOX9, HIC1, STAT4, TCF4, MYC, JUN, IRF7, E2F1, GLI1, CREB5  
5 STAT1) and concomitantly subjected or not to TGF $\beta$ 1 treatment. The RTqPCR were normalized with TUBB and the mock siRNA treated (TGF-b) condition was set to one. The treatments of NHDFs with siRNA against SRF, HIC1 or STAT4 lead to extensive cell death (\*): no analysis was possible. The treatments of NHDFs with siRNA against STAT1 and  
10 USF2 represent a control experiment as these factors do not have any action on fibroblast to myofibroblast differentiation.

**Figure 2b:** Graphic representation of the percentage of differentiated cells as assessed by the percentage of alpha SMA positive cells after treatment of NHDFs as described in a). The treatments of NHDFs with siRNA against SRF, HIC1 or STAT4 lead to extensive cell death  
15 (\*): no analysis was possible. The treatments of NHDFs with siRNA against STAT1 and USF2 represent a control experiment as these factors do not have any action on fibroblast to myofibroblast differentiation.

**Figure 2c:** Graphic representation of the  $\alpha$ SMA mRNA levels as assessed by RT-qPCR. Primary human dermal fibroblasts were treated either with mock siRNA or siRNA directed  
20 against different mRNA (PPARG, MAF, MEOX2, SIX2, STAT1 or USF2). The RTqPCR were normalized with TUBB and the mock siRNA treated (TGF-b) condition was set to 1. The treatments of NHDFs with siRNA against STAT1 and USF2 represent a control experiment as these factors do not have any action on fibroblast to myofibroblast differentiation.

**Figure 3:** short and long timing after TGF $\beta$ 1 treatment of FOXS1, EGR2, SRF, SOX9, HIC1, STAT4, TCF4, MYC, JUN, IRF7, E2F1, GLI1, CREB5. For each Factor, graphic representation of the mRNA levels after increasing time of treatment of the NHDF with TGF $\beta$  as assessed by RT-qPCR.  
25

**Figure 4:** short and long timing after TGF $\beta$ 1 treatment of PPARG, SIX2, MEOX2, MAF. For each Factor, graphic representation of the mRNA levels after increasing time of treatment of the NHDF with TGF $\beta$  as assessed by RT-qPCR.  
30

**Figure 5** Graphic representation of the TCF4 (a), EGR2 (b), SOX9 (c), STAT4 (d), FOXS1 (e), PPARG (f), MAF (g), MEOX2 (h), and SIX2 (i) mRNA levels after NHDF (donor A) treatment for 48h (light grey) or 72h (dark grey) either with mock siRNA or siRNA directed against different TF mRNA (SOX9, EGR2, TCF4, or FOXS1) and concomitantly treated with TGF- $\beta$ . For all graphs, the mock siRNA treated with TGF- $\beta$  (T+E) condition was set to 100% for each time of treatment (48h and 72h).

**Table 1:** gene to increase or decrease to treat and/or prevent fibrosis, hypertrophic scars or keloids

**Table 2:** siRNA sequences against the target genes

## 10 EXAMPLE

In response to a lesion, fibroblasts migrate into the wound where they differentiate into contractile myofibroblasts that will finally enter into apoptosis during the remodeling phase. This differentiation process can be studied ex-vivo in environmentally controlled tissue culture conditions, and therefore the timely controlled succession of different gene expression patterns can be addressed.

## Materials and methods

### Establishment of an ex vivo model of fibrosis, hypertrophic scar or keloid

Myofibroblasts represent the key players in the physiological reconstruction of skin after injury and in generating the pathological tissue deformations that characterize fibrosis such as hypertrophic scars (Desmouliere A, Chaponnier C, Gabbiani G (2005) Tissue repair, contraction, and the myofibroblast. Wound Repair Regen 13: 7-12).

To study the myofibroblasts involved in generating fibrosis, hypertrophic or keloid scars, NHDF were cultivated on collagen coated culture plates in DMEM-F12 (Invitrogen), supplemented with 10% FCS (Invitrogen), 5Ng/mL of insulin and 1ng/mL of b-FGF (PromoKine) and 10 ng/mL of TGF-O1 (Promocell), as TGF-O1 is known to induce the expression of  $\alpha$ SMA in fibroblasts (Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G 13 (1993) Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol, 1993 jul, 122(1): 103-111).

The efficiency of fibroblast differentiation was estimated by analyzing the expression of the myofibroblast marker alpha smooth muscle actin ( $\alpha$ SMA).

This  $\alpha$ SMA expression was assessed by RT-qPCR (mRNA levels) and by Western Blot (protein).

- 5 The efficiency of fibroblast differentiation was estimated by analyzing the expression of the myofibroblast marker alpha smooth muscle actin ( $\alpha$ SMA).

#### Western Blotting assay

Total proteins were extracted by scratching the cells with lysis buffer (TRIS, NaCl, NP40, EDTA, IMDTT) and incubated 30 min in ice. To remove cell debris, the samples were  
10 centrifuged at 13,000 x g for 10 min at 4°C and store at -20°C until use. Protein concentration was determined according to BCA method (Sigma). Equal amounts of total protein (20 $\mu$ g) were loaded to NuPAGE 10% BIS-Tris gel (Invitrogen), separated by migration at 150 V, and transferred to nitrocellulose membrane (Whatman) 1 hour at 30 V. Then, membranes were stained for  $\alpha$ SMA (Abcam) and tubulin (Abcam). Incubations were followed by secondary  
15 antibodies goat anti-rabbit IgG and goat anti-mouse IgG, respectively, conjugated with horseradish-peroxidase (HRP) (Promega). Signals were detected by ECL chemiluminescence using UptiLight HS WB Substrate (Uptima, Interchim). Bands were digitized with a scanner and the ratio between all bands density of the same blot was calculated by software (ImageJ 1.43u, 64-bit). Relative  $\alpha$ SMA expression was normalized to the respective value for tubulin.

#### 20 Total RNA Sample Preparation

After four days of experiment, treated fibroblasts were lysed with TRIzol Reagent (Invitrogen) and stored at -80°C. Then RNA was purified using chloroform and precipitated by isopropanol. Total RNA was quantified on the NanoDrop 2000c Spectrophotometer (Thermo Scientific). Reverse transcription of 500 ng total RNA to cDNA was done with  
25 oligot dT (Invitrogen) using SuperScript III RT (Invitrogen) and RNase OUT (Invitrogen). The cDNA was store at -20°C.

#### Quantitative real-time RT-PCR

Quantitative real-time PCR (RT-qPCR) was done using 5 $\mu$ L of 1:20 diluted cDNA on the LightCycler480 system (Roche) using Maxima SYBR Green qPCR Master Mix (Fermentas).  
30 Forward and reverse primers were designed by Eurofins (MWG,  $\alpha$ SMA forward: CTGTTTTCCCATCCATTGTG (SEQ ID NO:9),  $\alpha$ SMA reverse:

CCATGTTCTATCGGGTACTT (SEQ ID NO:10)) and a 100 $\mu$ M stock was stored at -20°C. Forward and reverse primer pairs were used for each RT-qPCR reaction. The cycling conditions were as follows : an initial 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 sec, 58°C for 30 sec, 72°C for 20 sec. LightCycler 480 SW 1.5 was used to evaluate the  
5 TM curves, to determine the C<sub>p</sub> and to approximate the relative concentration for each amplification reaction.

#### siRNA treatment

The expression the different TF was knocked down by transiently transfecting NHDF with specific small interfering RNAs. At least two different siRNAs (Qiagen) or highly specific  
10 ON TARGET PLUS smartpool siRNAs (Thermo Scientific) were used for each target. NHDF were treated with 10nM siRNA and 4 $\mu$ L of INTERFERin reagent (PolyPlus, Ozyme), according to the manufacturer's instruction and concomittanty treated with TGF- $\beta$ 1 and exudates for 6 days. To maintain a sufficient knocking down, a second transfection was performed at 48h after the first one. The efficiency of the knock down was assessed 48h after  
15 the first transfection.

#### $\alpha$ -Smooth Muscle Actin Immunofluorescence

Cells grown in collagen coated culture dishes, and treated as previously described, were fixed with 4% paraformaldehyde (PFA) in PBS for 15 minutes and permeabilized with 2.5% Triton  
20 X-100 (Euromedex, 2000-B) in PBS for 3 minutes. After saturation with 5% BSA in PBS, cells were stained for  $\alpha$ -SMA (Abcam, ab5694) and for DNA (DAPI). As secondary antibody, CyTM3 conjugated anti rabbit (GE Healthcare, PA43004) was used. Samples were observed with an oil immersion objective (Plan Fluor 40X/1.30 Oil, Nikon) on a Nikon ECLIPSE Ti (Nikon). Digital images were taken with a digital camera (Cool SNAP HQ<sup>2</sup>, Photometrics)  
25 and software (MetaMorf 7.5.4.0). To estimate the percentage of fibroblast differentiation due to the different treatments, the total number of cells per field was determined by the DAPI, and myofibroblasts, differentiated fibroblasts, were counted using the  $\alpha$ -SMA staining. Then, STUDENT (t-) and  $\chi^2$  tests were realized to evaluate the differentially expression of  $\alpha$ SMA between the untreated fibroblasts (without TGF $\beta$ ) and the treated ones.

#### 30 Network analysis

In order to enlighten master regulators of fibroblast fate after each different treatment, we have performed a gene network analysis treating gene expression lists determined after mRNA seq deep sequencing analysis of the gene profile of fibroblasts treated with TGF  $\beta$  with the gene profile of fibroblast treated without TGF $\beta$ . In these analysis and based on the assumption that the decrease or increase of interconnected genes is of stronger significance than a significant Log FC, we have used lists of genes selected only based on their P value and not on the value of their Log FC. We have performed two types of analysis: an ingenuity “upstream regulator analysis” and a DIRE (<http://dire.dcode.org/>) analysis. The Ingenuity “upstream regulator analysis”, given the particular profile of genes expression between two conditions, consists in selecting potential upstream regulators. The DIRE analysis is based on the selection of potential common regulatory elements between genes based on these elements conservation during evolution. From these identified elements, DIRE is able to provide a list of master regulators for a list of co-regulated genes. From those two analyses, and for each list analyzed, we have selected Transcription Factors (TFs) expressed in at least one of the two conditions considered in the concerned list (i.e. number of sequencing his superior to twenty in at least one of the two conditions). Then, we have deeply compared the two sets of analysis and decided to keep in the “key regulators lists” transcription factors belonging to both analyses. Because of possible bias in these two analyses we also decided to rescue transcription factors belonging only to one analysis and not the other but presenting very interesting target genes pattern in one list or the other. Altogether, these genes networks analysis allowed us to propose a list of TFs being key regulators in one or the other fibroblast fate (Fig 1)

### **Gene expression route upon fibroblast to myofibroblast differentiation**

Identification of the main molecular targets implicated in fibroblast differentiation of human primary fibroblasts under normal and pathological conditions

We have performed an in silico gene network analysis to enlighten putative upstream regulators of the different gene expression routes defined previously. This approach was original in the sense that we used global gene network analysis to identify potential key regulators and we did not take into account a change in these factors expression to select them. For example, we used the DIRE program (<http://dire.dcode.org/>) to identify evolutionary conserved potential regulatory elements in the different genes lists which allowed us to enlighten transcription factors that could potentially bind to these elements and

thus regulate these sets of genes. Twenty-three transcription factors were selected out from this analysis.

To prioritize the extensive study of the different Transcription Factors (TFs), we performed a time response study of TFs after the different fibroblast treatments. We did a short (between 5 30mn and 8 hours) and a long (between 8 hours and 96 hours) analysis of their changes in expression after the different treatments (Fig 3 and 4).

We have performed an exhaustive siRNA-based approach to study in one hand the role of these different factors in normal fibroblast to myofibroblast differentiation pathway (Figures 2).

10 The siRNA knock-down of fourteen of the potential key transcription factors identified therein inhibited the fibroblast to myofibroblast differentiation pathway as assessed by analyzing the  $\alpha$ SMA expression from TGF $\beta$  and siRNA-treated NHDFs: GLI1, HIC1, TCF4, SOX9, STAT4 MYC, CREB5, IRF7, JUN, E2F1, EGR2, SRF, FOXS1 as their knockdown decreased inhibit more or less efficiently the myofibroblast differentiation (Fig. 2 a-b-c). Very 15 interestingly, except for SOX9, FOXS1 and EGR2 which expression is strongly and rapidly up regulated upon TGF $\beta$  treatment, the mRNA levels of the other factors is constant during the first day or so after TGF $\beta$  treatment and overall unchanged during the four days of differentiation. This indicates that the maintenance of their expression but not their over-expression is necessary for fibroblast to myofibroblast differentiation.

20 The siRNA knock-down of four other potential key transcription factors identified by the in silico analysis (MAF, SIX2, MEOX2 and PPARG) seemed to induce the fibroblast to myofibroblast differentiation in absence of TGF $\beta$  to the same extend as the one obtained with mock transfected cells treated with TGF $\beta$ . Altogether these results showed that with knocking-down approaches we were able either to reduce or induce fibroblast to 25 myofibroblast differentiation (Table 1).

An in silico gene network analysis allowed us to identify potential key regulators of fibroblast cell fate either during differentiation into. By knocking down approaches, we found a strong effect on differentiation for nineteen of these factors.

We have also identified factors which seemed to play a role but maybe not as strongly as the 30 ones described in the paragraph before as their knockdown leads to consistent but mild decrease of  $\alpha$ SMA expression. These factors are MYC, JUN, E2F1, IRF7 and CREB5.

Very interestingly, we showed that the inactivation of some transcription factors leads to an increase of fibroblast differentiation per se. The knocking down of PPARG mRNA leads to an increase of fibroblast to myofibroblast differentiation.

5 FOXS1 belongs to the forkhead family of transcription factor often involved in developmental processes such as morphogenesis and differentiation. It has been shown that FOXS1 is of primary importance in the development of testicular vasculature. Moreover, FOXS1 was described as an early sensory neuronal marker. Here we show that inactivation of FOXS1 leads to an increase of myofibroblast differentiation in absence of TGF $\beta$ .

10 MEOX2 has already been described as implicated in TGF $\beta$  pathway as it was identified as an important factor in cleft palate development in TGF $\beta$ 3 knockout mice. Experiments in C2C12 myoblast cells showed that MEOX2 is also important for skeletal muscle development and differentiation. Here, we showed that siRNA directed against MEOX2 lead to a bypass of the exudate effect by fibroblasts to be able to differentiate into myofibroblasts.

15 In T cells, it has been shown that MAF was responsible for inhibition of IL22 expression by neutralizing TGF $\beta$ . TGF $\beta$  and MAF have antagonist/opposite effects on IL21 expression in CD4(+) T cells. In the same connection, in this study, we implicated MAF as an inhibitor of fibroblast to myofibroblast differentiation in absence or presence of exudate as its inactivation by siRNA leads to an increase of myofibroblast differentiation. On the contrary, during chondrocyte differentiation, a long form of MAF interacts and cooperates with SOX9 to  
20 activate downstream targets. This is another example of the differences between myofibroblast and chondrocyte differentiations.

SIX2 has been involved in maintaining pluripotency in kidney: in embryonal renal mesenchyme cells it is able to suppress differentiation and during kidney development it maintains the progenitor pool. Here, in dermal fibroblast, the invalidation of SIX2 leads to a  
25 bypass of the dominant exudate effect on TGF $\beta$  signaling.

### **Regulatory interactions between key transcription factors during fibroblast to myofibroblast differentiation**

The expression of TCF4 mRNA was not modified after SOX9, EGR2 or FOXS1 knockdown (Fig 5) placing it at the top of the regulatory interaction network between these factors. On the  
30 contrary EGR2 expression was largely inhibited upon siRNA treatment against TCF4 and SOX9 but remained unchanged upon treatment against FOXS1 (Fig 5) placing it after TCF4

and SOX9 but before FOXS1 in the network. SOX9 mRNA remained largely unchanged upon TCF4 and FOXS1 knockdown. We placed FOXS1 as a downstream target of SOX9. On the contrary SOX9 is upregulated upon EGR2 knockdown (Fig 5) The STAT4 mRNA is downregulated by TCF4, SOX9 and EGR2 knockdown (Fig 5) placing it as a downstream target of those TF whereas it remained unchanged upon FOXS1 siRNA treatment placing it beforehand. Consistently, FOXS1 mRNA is downregulated upon TCF4, SOX9 and EGR2 siRNA treatment (Fig 5) placing it at the end of this cascade. Globally, the PPARG, MAF and MEOX2 mRNA were upregulated upon siRNA treatment against TCF4, SOX9, EGR2 and FOXS1 (Fig 5) consistent with their role as antagonist in fibroblast differentiation. SIX2 mRNA level was unchanged upon EGR2 and FOXS1 knockdown (Fig 5) but upregulated in the same manner by TCF4 and SOX9 consistent with a close interconnexion between these two TF and suggesting the existence of a balanced signal between the differentiation agonists SOX9 and TCF4 and the antagonist SIX2.

15 The identification of transcription factors able to bypass differentiation is of major importance in the fibrosis, hypertrophic scar or keloid. In this study, by focusing on fibroblast, by no mean we tried to dissimulate the importance of other cells like neutrophils and macrophages in the skin healing process but we willingly simplified the biological context to draw a clearer picture of the situation.

## 20 Cancer connection

Myofibroblast are not only key cells in fibrosis, their importance has long been proven in cancer too where the presence of myofibroblast in the stroma as “cancer associated fibroblast” facilitate tumor development and has been often associated with inflammation, cell invasion, high grade malignancy and poor prognosis. Myofibroblast are enriched in stroma from prostate cancer compared to benign prostatic hyperplasia and associated at the leading edge of carcinomas. They produce MMP, cytokines (IL8, VEGF) and chemokines (CXCL12) to promote cancer proliferation, tumor invasion and neo-angiogenesis. As alternative therapeutic tracks, it seems of primary importance to target stromal cells (and among them myofibroblasts) in cancer to diminish its abilities to migrate, invade and proliferate. But the cancer case is not so trivial; it has been suggested that, as inflammation is often linked to cancer, finding new ways of bypassing or inhibiting inflammation could also help fighting against cancer. This extensive study on TGF $\beta$ -dependent fibroblast to myofibroblast

differentiation gives a lot of new paths for therapeutic targets discovery. Nonetheless, because of TGF $\beta$  dual pro-fibrosis and anti-inflammation activities, therapeutics playing with inhibition or re-enforcement of TGF $\beta$  action should be well-balanced as inhibition of fibrosis via inhibition of TGF $\beta$  pathway could also increase cancer susceptibility.

- 5 The better understanding of normal fibroblast to myofibroblast differentiation is of major importance in the field as it opens new ways into novel therapeutics markers or targets that can be used in other important pathologies.

Tissue fibrosis (like pulmonary fibrosis, liver fibrosis, renal interstitial fibrosis, cardiovascular fibrosis, hypertrophic or keloid scars...) is primarily attributed to an excessive activation of  
10 ECM producing myofibroblasts by TGF $\beta$ 1. A better understanding of the gene expression route from fibroblast to myofibroblast is of great interest in the fibrosis understanding and curing. We showed that exudate from chronic wounds are able to prevent and reverse the myofibroblast differentiation giving new signs of the reversibility of differentiation as it has already been shown for liver, kidney or Hepato Stellar Cell (HSC) derived myofibroblast. We  
15 also identify transcription factors which inactivation inhibits TGF $\beta$ -dependent myofibroblast differentiation. Myofibroblasts are not only key cells in fibrosis, their importance has been long proven in cancer too where the presence of myofibroblast in the stroma as “cancer associated fibroblast” facilitate tumor development and has been often associated with inflammation, cell invasion, high grade malignancy and poor prognosis. Myofibroblast are  
20 enriched in stroma from prostate cancer compared to benign prostatic hyperplasia and associated at the leading edge of carcinomas. They produce MMP, cytokines (IL8, VEGF) and chemokines (CXCL12) to promote cancer proliferation, tumor invasion and neo-angiogenesis. As alternative therapeutic tracks, it seems of primary importance to target stromal cells (and among them myofibroblasts) in cancer to diminish its abilities to migrate,  
25 invade and proliferate. But the cancer case is not so trivial; it has been suggested that, as inflammation is often linked to cancer, finding new ways of bypassing or inhibiting inflammation could also help fighting against cancer. The study on TGF $\beta$ -dependent fibroblast to myofibroblast differentiation shows a lot of new paths for therapeutic targets discovery. Nonetheless, because of TGF $\beta$  dual pro-fibrosis and anti-inflammation activities,  
30 therapeutics playing with inhibition or re-enforcement of TGF $\beta$  action should be well-balanced as inhibition of fibrosis via inhibition of TGF $\beta$  pathway could also increase cancer susceptibility.

**Claims**

1. A therapeutic compound comprising :

5 - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4;

and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

10 for use in the prevention and/or treatment of fibrosis, hypertrophic scar or keloid.

2. A therapeutic compound according to claim 1 further comprising:

- an agent that inhibits the activity of at least one gene selected from the group consisting of E2F1, EGR2, GLI1, JUN, MYC, SMAD3, SMAD4, SOX9, SRF preferentially EGR2, SOX9

15 and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of ETS1 and PPARG.

3. A therapeutic compound according to claim 1 or 2 wherein said agent is selected from the group consisting of: anti-sense DNA or RNA, siRNA, shRNA, cDNA, TALENS or ribozymes, either naked or in the form of plasmid or viral vectors.

20

4. A therapeutic compound according to claim 1 or 2 wherein said agent is an inhibitor or enhancer of protein function.

5. A therapeutic compound according to claim 4 wherein said agent is selected from the group consisting of: a binding agent that binds, either reversibly or irreversibly, to inhibit protein function such as an antibody or a synthetic antagonist; or an agent that works upstream or downstream of the protein signaling mechanism to inhibit protein function.

25

6. A therapeutic compound according to any preceding claim wherein the therapeutic compound is for use in treating mammalian fibrosis, hypertrophic scars or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid.

7. A therapeutic compound according to any preceding claim wherein the therapeutic compound is for use in treating human fibrosis, hypertrophic scars or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid
8. A therapeutic compound according to any preceding claim wherein the therapeutic  
5 compound is for topical application.
9. A therapeutic compound according to any preceding claim wherein the therapeutic compound is for application to a dressing or impregnation of a dressing.
10. A pharmaceutical composition comprising a therapeutic compound according to any preceding claim together with a pharmaceutically acceptable carrier.
- 10 11. A method for preparing a pharmaceutical composition according to claim 10 comprising bringing said therapeutic compound in conjunction or association with a pharmaceutically or veterinary acceptable carrier or vehicle.
12. A method for treating a mammalian fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid wherein said method comprises  
15 administering to said fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid a therapeutic compound comprising:
- an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4
- 20 and/or
- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2.
13. A kit for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid wherein said kit comprises:
- 25 (a) at least one therapeutic compound according to any of claims 1 -9 or a composition according to claim 10 and
- (b) at least one dressing for applying to said fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid.

14. A combination therapeutic for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid comprising:

(a) - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4,  
5 preferentially TCF4, FOXS1, STAT4

and/or

- an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

and

10 b) at least one further therapeutic.

15. Use of - an agent that inhibits the activity of at least one gene selected from the group consisting of FOXS1, CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, TCF4, preferentially TCF4, FOXS1, STAT4

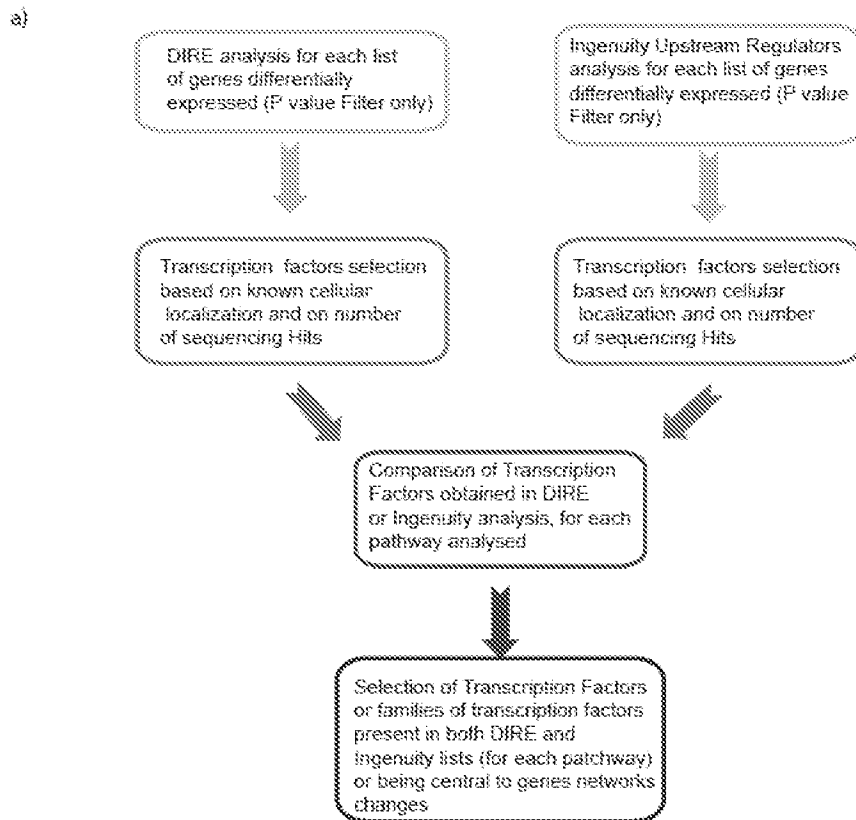
and/or

15 - an agent that enhances the activity of at least one gene selected from the group consisting of MAF, MEOX2, SIX2

for treating a fibrosis, hypertrophic scar or keloid or tissue susceptible of developing fibrosis, hypertrophic scar or keloid

wherein said agent modulates fibroblast and myofibroblast differentiation and/or activity

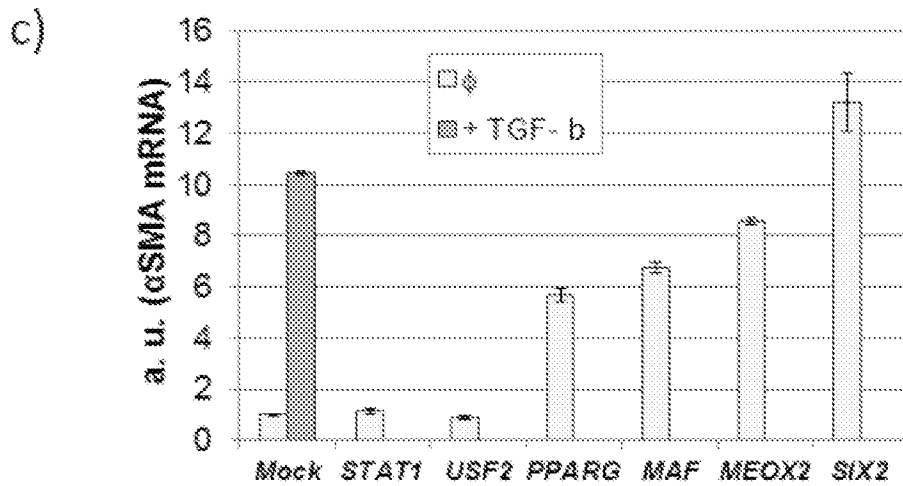
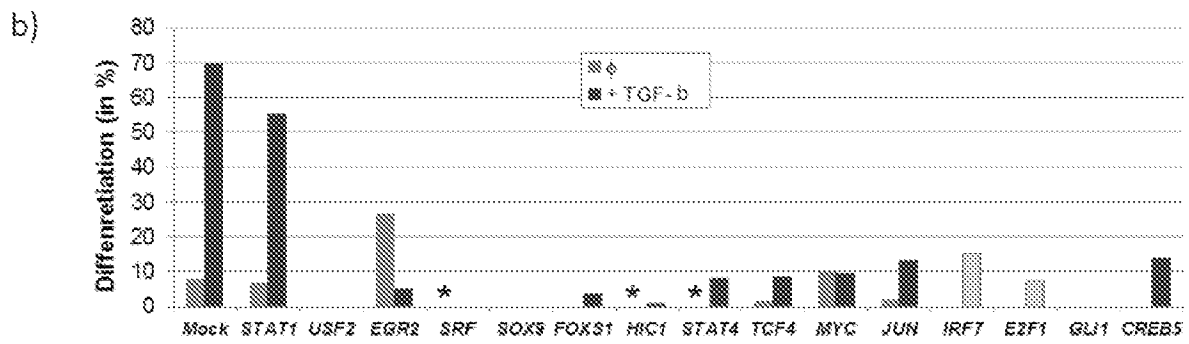
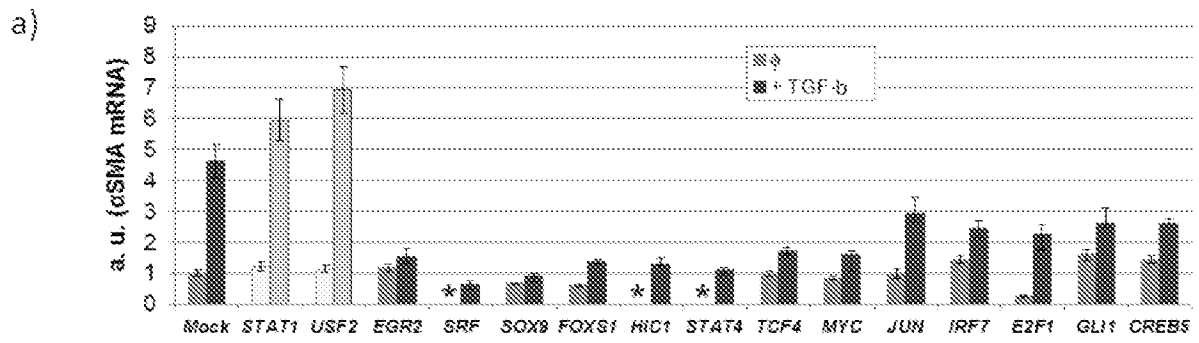
20



b)

Symbol	Entrez Gene Name
CREB5	cAMP responsive element binding protein 5
E2F1	E2F transcription factor 1
EGR2	early growth response 2
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
FOXS1	forkhead box S1
GLI1	GLI family zinc finger 1
HIC1	hypermethylated in cancer 1
IRF7	interferon regulatory factor 7
JUN	jun oncogene
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
MEOX2	mesenchyme homeobox 2
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
POU2F2	POU class 2 homeobox 2
PPARG	peroxisome proliferator-activated receptor gamma
SIX2	SIX homeobox 2
SMAD3	SMAD family member 3
SMAD4	SMAD family member 4
SOX9	SRY (sex determining region Y)-box 9
SRF	serum response factor (c-fos serum response element-binding transcription factor)
STAT1	signal transducer and activator of transcription 1 91kDa
STAT4	signal transducer and activator of transcription 4
TCF4	transcription factor 4
USF2	upstream transcription factor 2 c-fos interacting

Figure 1



Figures 2

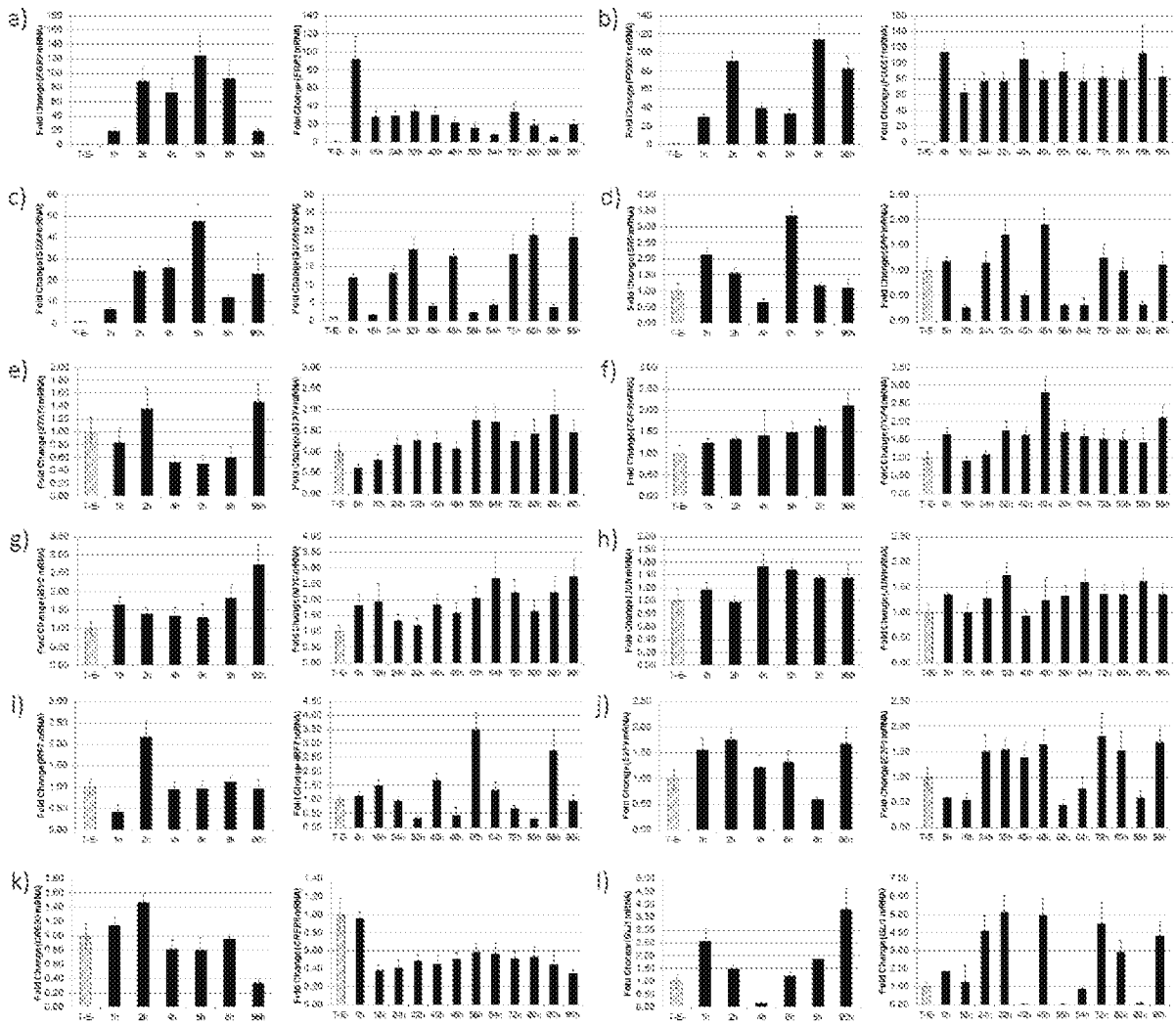


Figure 3

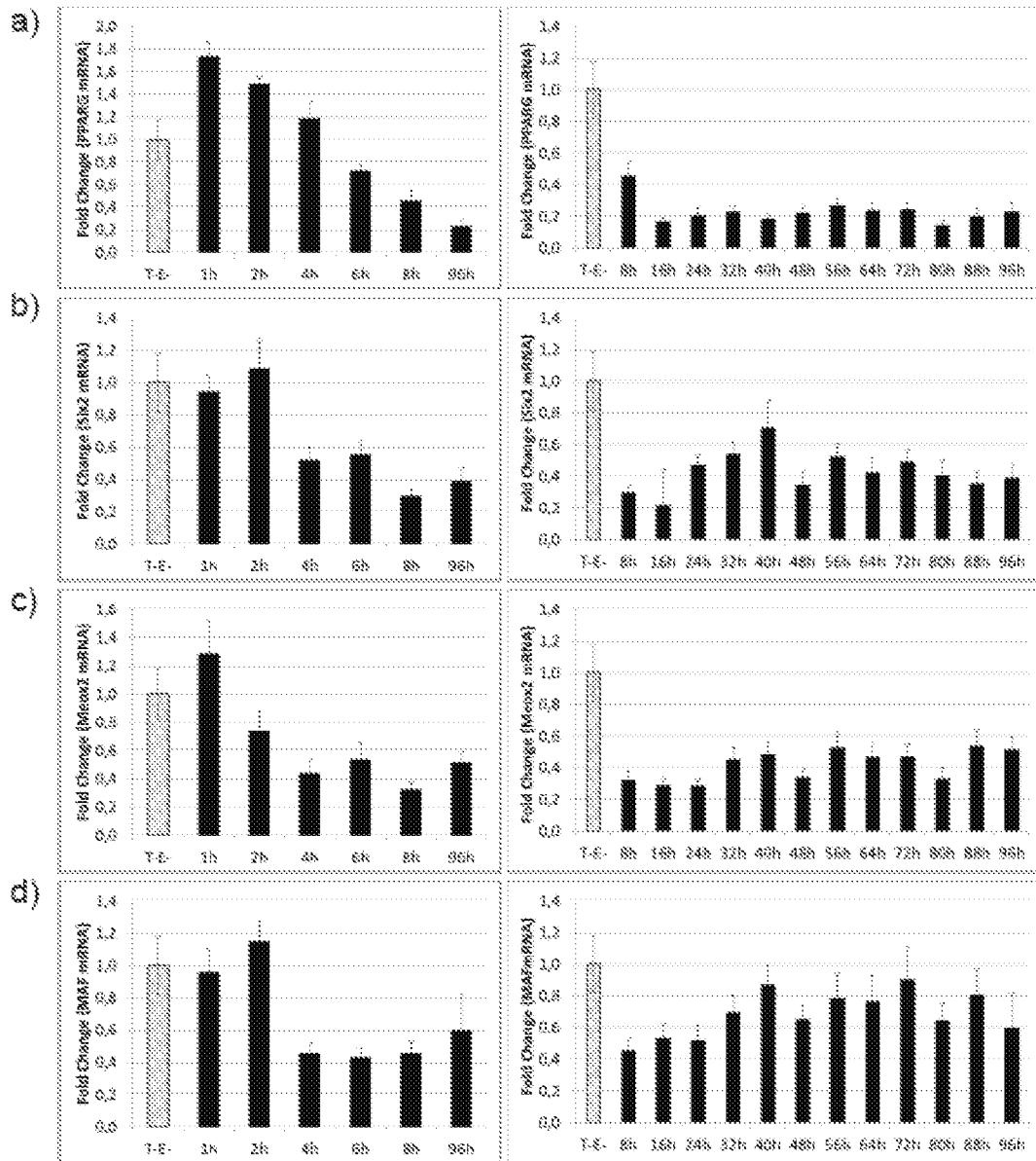


Figure 4

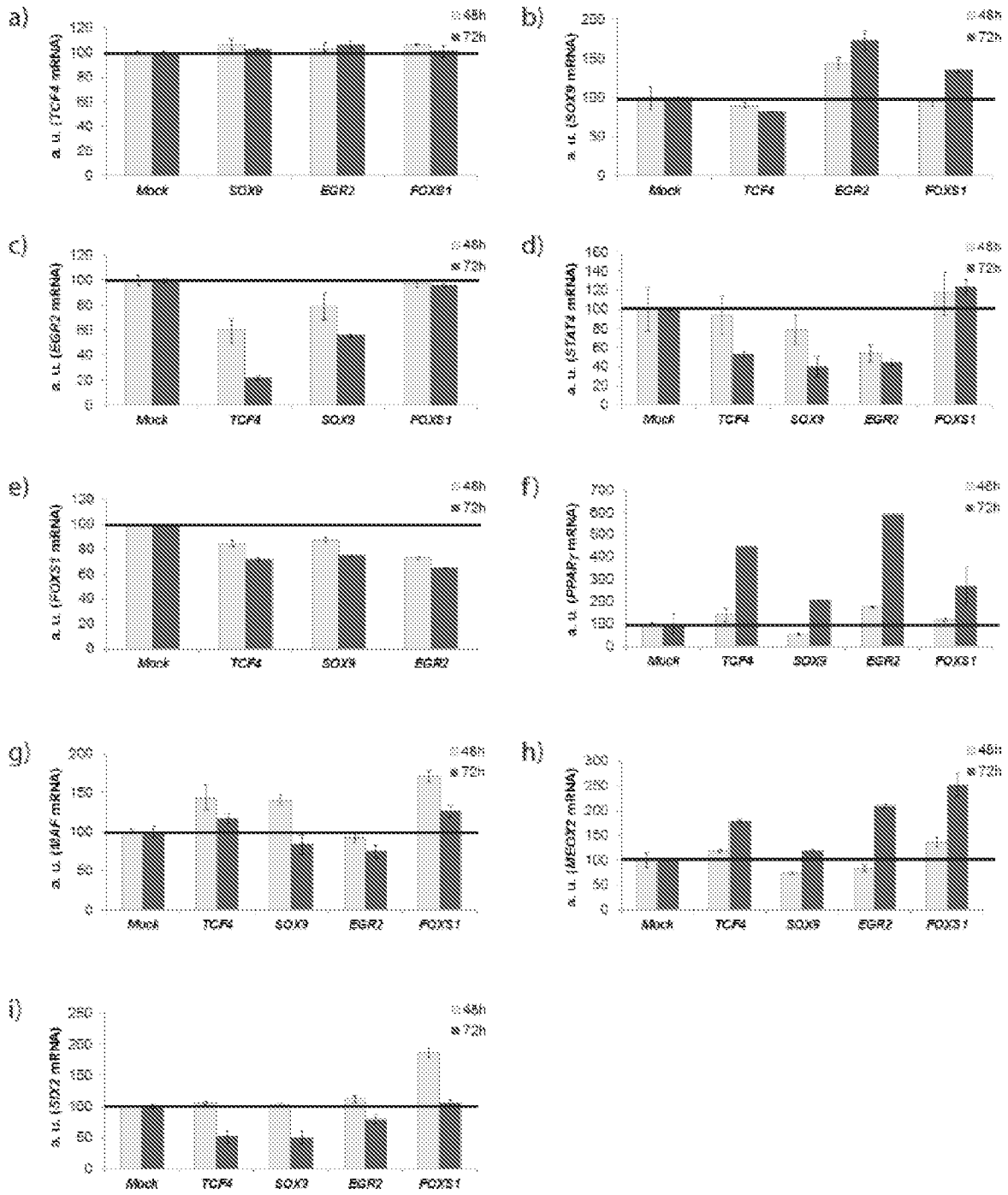


Figure 5

CREB5	To decrease
E2F1	To decrease
EGR2	To decrease
ETS1	To increase
FOXS1	To increase
GLI1	To decrease
HIC1	To decrease
IRF7	To decrease
JUN	To decrease
MAF	To increase
MEOX2	To increase
MYC	To decrease
POU2F2	To decrease
PPARG	To increase
SIX2	To increase
SMAD3	To decrease
SMAD4	To decrease
SOX9	To decrease
SRF	To decrease
STAT1	To increase
STAT4	To decrease
TCF4	To decrease
USF2	To decrease

**Table 1**

CREB5	CCGGCGAAGGGUGGHAGACGA	Q. S104157174	11
	AACAGUAUUCUCUAGGAUCUA	Q. S104141899	12
E2F1	UCGGAGAAUUCAGAUUCU		13
	GAGAAGUCACGCUAUGAGA	T. L-003259-00	14
	GAGCAGAUUGUUAUGGUGA		15
	GAACAGGGCCACUAGCUCU		16
EGR2	GAAGGCALAAUCAAUAUUG		17
	CUACUGUGGCCGAAAGUUIU	T. L-006527-00	18
	BAAACCAGACCUUCACUUA		19
	GAGAAGAGGUGGUJGGAUU		20
FOXS1	ACGGCCAAHAAAGCCAUUGCA	Q. S104152540	21
	CHGGCUCUAGGACCHGGAAGAS	Q. S100387527	22
GLI1	GCAAAUAGGGCUUCACAUA		23
	AGGCUCAGCUUGUGUGUAA	T. L-003896-00	24
	GSACCAGGCACCUUSCAUU		25
	CAGCUAGAGUCAGAGGUU		26
HIC1	GCACAGCAAGCCAAACUCA		27
	GAGCUUCCGUGACAAGCUG		28
	UGAUCAUUGUUGGUGCAGAA		29
	GACCAUCGACCCUUCUCU	T. L-006532-00	30
IRF7	GCACAAGGUGUAGCGCCUC		31
	CAACUUCGCGUCGCGACUG	T. L-011810-00	32
	GCGGSCAUUUCAGGCCU		33
	CAGGCACGGACCCAGACUGA		34
JUN	GAGCGGACCUUAUGCCUAC		35
	GAACAGGUGGCACAGCUUA	T. L-003268-00	36
	GAACGACCUUCUAUSAGC		37
	UGAAAGCUCAGAAGUCCGA		38
MAF	UUCGAUCUGAUGAAGULUGAA	Q. S100079048	1
	CGCCAGGAGAAUGGCCALICAA	Q. S103086069	2
MEOX2	AGCAUUCGCAUUAUUSAUUA	Q. S104299421	3
	CCGCGCCGUCUUSUGUCCAA	Q. S104262174	4
MYC	ACGGAACUCUUGUGCGUAA		39
	GAACACACAAGCUCUUGGA	T. L-003282-00	40
	AACGUAGGULUCACCAACA		41
	CGAUGUUGUUCUUGUGCAA		42
PPAR $\gamma$	UCGGGAGAACAUUCAGAUUGA	Q. S103115286	5
	GAGCGGCAUCUUSACAGCAAA	Q. S100071873	6
SIX2	GAACGAGAAUCCAAUUCUAA	Q. S100719313	7
	CCGCCUGAAUUGGCAGCGCAA	Q. S104141095	8
SMAD3	CAACAGGAAUUGCAGCAGUG		43
	GAGUUCGCUUCAAUUAUA	T. L-020067-00	44
	GGACCGCAGGUUCUCCAAAC		45
	UUAGAGACAUCAGUAUGG		46
SMAD4	GCAAUUGCAAAGUUGGUAA		47
	CCGACAGCUUUAAGACUGA	T. L-003802-00	48
	GAUUCUUAUUCUACGAA		49
	GUACAGAGUUAUACUJAG		50
SOX9	GGAAACAAGCCGUCUACACA		51
	GAACAAGCCGCACGUCAAAG	T. L-021507-00	52
	GACCUUCGAGUUCAAAGAG		53
	GGAAGUCGUGAGAAGCCE		54
SRF	UGAGACAGGCCAUUGUUAU		55
	GCACUGUGCUGAAGAGUAC	T. L-009800-00	56
	GCACCAAGAGUGAAUGAUC		57
	GCACCAAGUUCUCUAGUG		58
STAT1	GCACCAUUGGCCUUCAGCUU		59
	CUACGAAUUAUAGCCUAUC	T. L-003543-00	60
	GAACCUAGCUUCUAGCCGG		61
	AGAAAGAGCUUGACAGUAA		62
STAT4	GAACUAAACUUCAGGUAA		63
	GCAUGUAGCUUGUGUUAUU	T. L-011784-00	64
	CAAUUCUAGCUACAGUUAUG		65
	CUGCCAGACUACAAGULIA		66
TCF4	GCACUUGCCUUCGUAUUAU		67
	GACAAAGAGCCUGAGUGAUU	T. L-004594-00	68
	GCACAGCUCUUGGUCUAG		69
	CAACCGCACAGCAGUUA		70
USF2	GCAAGAGCGGAGCGAGUAA		71
	GGAGGGACAAGAUAACAA	T. L-003618-00	72
	GAAGAGCCAGCACAACGA		73
	CAAAAUCCUUCAGCAAUIS		74

Table 2

# INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2013/001989

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K14/47 C12N15/113 A61K48/00 A61K31/7088  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBL

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/085274 A2 (CODA THERAPEUTICS INC [US]; DUFT BRADFORD J [US]) 9 July 2009 (2009-07-09) Whole document, especially the claims -----	1-15
A	JÉRÔME AVOUAC ET AL: "Inactivation of the transcription factor STAT-4 prevents inflammation-driven fibrosis in animal models of systemic sclerosis", ARTHRITIS & RHEUMATISM, vol. 63, no. 3, 25 March 2011 (2011-03-25) , pages 800-809, XP055112868, ISSN: 0004-3591, DOI: 10.1002/art.30171 the whole document ----- -/--	1-15

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 April 2014

Date of mailing of the international search report

08/07/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Kools, Patrick

## INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2013/001989

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>TAO LING ET AL: "Role of the JAK-STAT pathway in proliferation and differentiation of human hypertrophic scar fibroblasts induced by connective tissue growth factor.",  MOLECULAR MEDICINE REPORTS 2010 NOV-DEC, vol. 3, no. 6, November 2010 (2010-11), pages 941-945, XP002722998,  ISSN: 1791-3004  the whole document</p> <p style="text-align: center;">-----</p>	1-15
A	<p>JENNIFER M. HAHN ET AL: "Keloid-derived keratinocytes exhibit an abnormal gene expression profile consistent with a distinct causal role in keloid pathology",  WOUND REPAIR AND REGENERATION, vol. 21, no. 4, 1 July 2013 (2013-07-01), pages 530-544, XP055112583,  ISSN: 1067-1927, DOI: 10.1111/wrr.12060  the whole document</p> <p style="text-align: center;">-----</p>	1-15
A	<p>CEDERBERG A ET AL: "Chromosome Localization, Sequence Analysis, and Expression Pattern Identify FKHL 18 as a Novel Human Forkhead Gene",  GENOMICS, ACADEMIC PRESS, SAN DIEGO, US, vol. 44, no. 3,  15 September 1997 (1997-09-15), pages 344-346, XP004459173,  ISSN: 0888-7543, DOI:  10.1006/GENO.1997.4864  the whole document</p> <p style="text-align: center;">-----</p>	1-15

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2013/001989

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009085274 A2	09-07-2009	AU 2008343755 A1	09-07-2009
		CA 2710232 A1	09-07-2009
		EP 2252320 A2	24-11-2010
		JP 2011507860 A	10-03-2011
		US 2013184220 A1	18-07-2013
		WO 2009085274 A2	09-07-2009
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2013/001989

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15(partially)

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(partially)

Therapeutic compound comprising an agent inhibiting the activity of FOXS1 for use in the prevention and/or treatment of fibrosis, hypertrophic scars or keloids. Pharmaceutical composition comprising said therapeutic compound. Method for preparing the pharmaceutical composition. Method for treating mammalian fibrosis, hypertrophic scars or keloids by administration of the therapeutic compound. Kit for treating a fibrosis, hypertrophic scar or keloid wherein the kit comprises the therapeutic compound. A combination therapeutic for treating a fibrosis, hypertrophic scar or keloid comprising the therapeutic compound and a further therapeutic. Use of the therapeutic for treating a fibrosis, hypertrophic scar or keloid, wherein said therapeutic modulates fibroblast and myofibroblast differentiation and/or activity.

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2-8. claims: 1-15(partially)

As for subject 1, now for agents inhibiting the activity of CREB5, HIC1, IRF7, POU2F2, SIX2, STAT4, or TCF4.

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9-11. claims: 1-15(partially)

As for subject 1 now for agents enhancing the activity of MAF, MEOX2, or SIX2

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