# Targeting TGFβ signaling pathway in fibrosis and cancer

Karkampouna, S.

# List of abbreviations

| | |
|---|---|
| 3D | Three-dimensional |
| ACTA2, αSMA | Aorta smooth muscle actin α 2 |
| ACVR2A | ACTIVIN A receptor type IIA |
| ACVR2B | ACTIVIN A receptor type IIB |
| ACVRL (ALK) | ACTIVIN A receptor-like kinase |
| Ad | Adenovirus |
| AFP | α-fetoprotein |
| AKT | V-akt murine thymoma viral oncogene homolog |
| ALD | Alcoholic liver disease |
| ALDH1A1 | Aldehyde dehydrogenase 1 family, member A1 |
| ALK1Fc/ ACE-041 | Activin receptor-like kinase-1- fragment crystallised region |
| ALK5 | Activin receptor-like kinase-5 |
| AMH | Anti-Mullerian hormone |
| AMSH | SH3 domain of signal transducing adaptor molecule |
| AON | Antisense oligonucleotide |
| AP-1 | Activator protein 1 |
| ARKADIA | Ring finger protein 11 |
| BAM (BCL-XL) | BCL-2 associated agonist of cell death |
| BAMBI | BMP and ACTIVIN membrane-bound inhibitors |
| bFGF | Basic fibroblast growth factor |
| BIM; | BCL-2-like 11 |
| BMI-1 | B cell-specific Moloney murine leukemia virus integration site 1 |
| BMP | Bone morphogenetic protein |
| BMPR | BMP receptor |
| BRE | BMP-response-element |
| CAGA | Cytosine adenine guanine adenine |
| CASP3 | Cleaved caspase 3 |
| CBP (TRX) | cAMP response element-binding protein, Trithorax protein |
| $CCl_4$ | Carbon tetrachloride |
| CDC42 | Cell division cycle 42 |
| CDK | Cyclin-dependent kinases |
| CDK4 | Cyclin-Dependent Kinase 4 |
| CDKN1A (p21) | Cyclin-dependent kinase inhibitor 1A |
| CDKN1B (p27KIP1) | Cyclin-dependent kinase inhibitor 1B |
| CDKN2B (p15INK2B) | Cyclin-dependent kinase inhibitor 2B |
| cDNA | Complementary DNA |
| CFc | Control Fc |
| C-FOS | Cellular FBJ (Finkel-Biskis-Jinkins) Murine Osteosarcoma viral oncogene homolog |
| C-JUN | Cellular- ju-nana (Abbreviated from Japanese,the number 17); derived from the ASV 17 provirus. |
| Ck19 | Cytokeratin 19 |
| CMYC | V-myc avian myelocytomatosis viral oncogene homolog |

| | |
|---|---|
| **COL1A1** | **Collagen type 1 α 1** |
| **COL1A2** | **Collagen type 1 α 2** |
| **co-SMAD** | **Common mediator SMAD (SMAD4)** |
| **c-SRC** | **Avian sarcoma (Schmidt-Ruppin A2) viral oncogene** |
| **CTGF/CCN2** | **Connective tissue growth factor** |
| **CV** | **Central vein** |
| **DAPPER2** | **Dishevelled-binding antagonist of β-catenin** |
| **DCN** | **Decorin** |
| **DD** | **Dupuytren's disease** |
| **DDCt** | **Delta-delta cycle threhold algorithm** |
| **DNA** | **Deoxyribonucleic acid** |
| **DNDM** | **DNA demethylating complex** |
| **E1A** | **Adenovirus early gene1** |
| **E2F** | **Elongation factor-2** |
| **ECM** | **Extracellular matrix** |
| **EGF** | **Epidermal growth factor** |
| **EGF-CFC** | **EGF-like, cysteine-rich CRIPTO-FRL1-CRYPTIC** |
| **EMT** | **epithelial-to-mesenchymal transition** |
| **ERG** | **v-ets avian erythroblastosis virus E26 oncogene homolog** |
| **ERK** | **Mitogen-activated protein kinase 1** |
| **ERK** | **Extracellular signal-regulated kinase** |
| **FKBP12** | **Inhibitor FK506-binding protein** |
| **FN** | **Fibronectin** |
| **FOX** | **Forkhead Box** |
| **FOXH1** | **Forkhead Box H1** |
| **GADD34** | **Growth arrest and DNA damage protein** |
| **GADD45B** | **Growth arrest and DNA-damage-inducible 45 beta** |
| **GATA3** | **GATA binding protein 3** |
| **GC** | **Guanine cytosine** |
| **GDF** | **Growth and differentiation factors** |
| **GPI** | **Glucophosphatidylinositol** |
| **GRP-78** | **Glucose-related protein-78** |
| **GS** | **Glycine-serine** |
| **GSK3** | **Glycogen Synthase Kinase 3** |
| **GTPase** | **Guanosinetriphosphatase** |
| **HBV/ HCV** | **Viral hepatitis B/ C** |
| **HCC** | **Hepatocellular carcinoma** |
| **HDACs** | **Histone Deacetylases** |
| **HHT** | **Hereditary telangiectasia** |
| **HMGA2** | **High motility group AT-hook 2 protein** |
| **HSCs** | **Hepatic stellate cells** |
| **ID** | **Inhibitor of differentiation** |
| **IKBa** | **Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha,** |

| | |
|---|---|
| **I-SMAD** | **Inhibitory SMAD** |
| **JAG1** | **Jagged 1** |
| **JNK** | **C-Jun N-terminal kinase** |
| **JUNB** | **Jun B proto-oncogene** |
| **LAP** | **Latency-associated peptide** |
| **LEF** | **Lymphoid enhancer-binding factor** |
| **LLC** | **Large latency complex** |
| **LRP5** | **Leucine-rich protein 5** |
| **LTBP** | **Latent TGFβ binding protein** |
| **luc** | **Firefly luciferase** |
| **MAPK** | **Mitogen-activated protein kinase** |
| **MEK** | **MAPK/ERK** |
| **MET** | **Mesenchymal-to-epithelial transition** |
| **MFBs** | **Myofibroblasts** |
| **MH1, 2** | **MAD homology 1, 2 domain** |
| **miRNA** | **Micro ribonucleic acid** |
| **MMP** | **Matrix metalloprotease** |
| **mRNA** | **Messenger RNA** |
| **mTOR** | **Mammalian target of rapamycin** |
| **MYO-D** | **Myogenic differentiation antigen** |
| **NICD** | **Notch intracellular domain** |
| **NKX2.5** | **NK 2 homeobox 5** |
| **NR4A1** | **Nuclear Receptor Subfamily 4, Group A, Member 1** |
| **NT** | **Non targeting** |
| **OCT4** | **Octamer-binding transcription factor 4** |
| **ODN** | **Oligodeoxynucleotide** |
| **OSX** | **Osterix (Sp7 transcription factor)** |
| **p38** | **Mitogen-activated protein kinase 14** |
| **p53** | **tumor protein p53** |
| **PACE-4** | **Paired basic amino acid cleaving enzyme-4** |
| **PAI-1** | **Plasminogen activator inhibitor 1** |
| **PAR6** | **Par-6 family cell polarity regulator** |
| **PCa** | **Prostate cancer** |
| **PCNA** | **Proliferating nuclear antigen** |
| **PDGFβ** | **Platelet derived growth factor β polypeptide** |
| **PH3** | **Phosphorylated histone 3** |
| **PI3K** | **Phosphoinositole-3 kinase** |
| **PP1,2** | **Protein phosphatase** |
| **PTEN** | **Phosphatase and tensin homolog** |
| **PTM** | **Post translational modification** |
| **PV** | **Portal vein** |
| **qRT-PCR** | **Quantititative real time polymerase chain reaction** |
| **RAC** | **Rho Family, small GTP binding protein** |

| | |
|---|---|
| **RAF** | **Virus-induced rapidly accelerated fibrosarcoma** |
| **RAS** | **Retrovirus-associated DNA sequences** |
| **RBP-Jk** | **Recombination signal binding protein for immunoglobulin kappa J region** |
| **Ren** | **Renilla luciferase** |
| **RGM** | **Repulsive guidance molecule** |
| **RHO** | **Rhodopsin** |
| **RLU** | **Relative light units (luc/ ren)** |
| **R-SMAD** | **Receptor-activated SMAD** |
| **RTK** | **Receptor tyrosine kinase** |
| **RUNX** | **Runt-related protein** |
| **Ser** | **Serine** |
| **SHG** | **Second harmonic generation** |
| **shRNA** | **Short hairpin RNA** |
| **SKI** | **Avian sarcoma viral (v-ski) oncogene** |
| **SLC** | **Small latency complex** |
| **SMAD** | **Small mothers against decapentaplegic** |
| **SMI** | **Small molecule inhibitor** |
| **SMURF** | **SMAD specific E3 ubiquitin protein ligase** |
| **SNAIL** | **Snail family zinc finger protein,** |
| **SNON** | **SKI-related oncogene** |
| **SOX2** | **Sry-related HMG box protein 2** |
| **SP1** | **Specificity protein-1** |
| **SRE** | **SMAD-response element** |
| **SWI/SNF** | **SWItch/Sucrose non-fermentable** |
| **TAK1** | **TGFβ-associated kinase 1** |
| **TDGF1** | **Teratocarcinoma-derived growth factor** |
| **TGFa** | **Transforming growth factor alpha** |
| **TGFβ** | **Transforming growth factor β** |
| **TGIF** | **TGFβ-induced homeobox factor** |
| **Thr** | **Threonine** |
| **TIMPs** | **Tissue inhibitor of metalloproteinase 1** |
| **TMPRSS2-ERG** | **transmembrane protease, serine 2** |
| **TOPRO** | **TO-PRO-3 nuclear dye** |
| **TP53** | **tumor protein p53** |
| **TPF** | **Two photon excited fluorescence** |
| **TRAF6** | **TNFα-associated factor 6** |
| **TSP-1** | **Thrombospondin-1** |
| **TWIST** | **Twist family BHLH transcription factor** |
| **Tyr** | **Tyrosine** |
| **TβRI** | **Transforming growth factor β receptor type I** |
| **TβRII** | **Transforming growth factor β receptor type II** |
| **VEGF** | **Vascular endothelial growth factor** |
| **ViM** | **Vivo-morpholino** |

| | |
|---|---|
| **WNT** | **Wingless-Type factor** |
| **X** | **any aminoacid** |
| **ZEB** | **zinc finger E-box binding homeobox** |
| **ZO-1** | **Tight junction protein 1** |

## Curriculum Vitae

Sofia Karkampouna was born on 3 December, 1986 in Ioannina, Greece. In 2009 she obtained her Bachelor's diploma in Molecular Biology and Genetics at Democritus University in Alexandroupoli, Greece. During 2009-2011 she followed the MSc program of Molecular Medicine in the Erasmus Medical Centre, Rotterdam, the Netherlands.
During her first research internship at the Cell Biology department she worked with Dr. Mihaela Crisan in Prof. Dr. Elaine Dzierzak's group focusing on embryonic hematopoietic stem cell regulation by BMP and Hedgehog signaling pathways. Her second internship (MSc thesis) was performed at the Cell Biology department in the group of Dr. Raymond Poot, focusing on the identification of interaction partners of neural transcription factors. In September 2011 she started her PhD studies at the department of Molecular Cell Biology in Leiden University Medical Centre, in the group of Prof. Dr. Peter ten Dijke and under the supervision of Dr. Marianna Kruithof-de Julio. Since September 2015 she is appointed at the department of Urology in Leiden University Medical Centre working on preclinical *ex vivo* models of fibrosis.

## List of publications

1. ALK1Fc suppresses tumor growth by impairing angiogenesis and proliferation of human prostate cancer cells *in vivo*.
Zoni E.,* **Karkampouna S.,*** Gray P., Goumans MJTH., Hawinkels L., van der Pluijm G., ten Dijke P and Kruithof-de Julio M. * equal contribution. *Submitted*

2. Human Dupuytren's *ex vivo* culture for the study of myofibroblasts and extracellular matrix interactions.
**Karkampouna S.,** Kloen P., Obdeijn MC., Riester SM., van Wijnen AJ., Kruithof-de Julio M.
J Vis Exp. 2015 Apr 18;(98). PMID:25938583

3. Inhibition of TGFβ type I receptor activity facilitates liver regeneration upon acute $CCl_4$ intoxication in mice.
**Karkampouna S.,** Goumans MJTH., ten Dijke P., Dooley S., Kruithof-de Julio M.
Arch Toxicol. 2015 Jan 8. PMID:25566828

4. Fibrosis: a novel approach for an old problem.
**Karkampouna S.,** Kruithof-de Julio M.
Receptors & Clinical Investigation 2014 June;1:e151. Editorial Review

5. Novel *ex vivo* culture method for the study of Dupuytren's disease: effects of TGFβ type I receptor modulation by antisense oligonucleotides.
**Karkampouna S.,** Kruithof BPT., Kloen P., Obdeijn MC., van der Laan AM., Tanke HJ., Kemaladewi DU., Hoogaars WM., 't Hoen PA., Aartsma-Rus A., Clark IM., ten Dijke P., Goumans MJTH., Kruithof-de Julio M.
Mol Ther Nucleic Acids. 2014 Jan 21;3:e142. PMID:24448195

6. BMP7 activates brown adipose tissue and reduces diet-induced obesity only at subthermoneutrality.
Boon MR., van den Berg SA., Wang Y., van den Bossche J., **Karkampouna S.,** Bauwens M., De Saint-Hubert M., van der Horst G., Vukicevic S., de Winther MP., Havekes LM., Jukema JW., Tamsma JT., van der Pluijm G., van Dijk KW., Rensen PC.
PLoS One. 2013 Sep 16;8(9):e74083. PMID: 24066098

7. TGFβ signaling in liver regeneration.
**Karkampouna S.,** ten Dijke P., Dooley S., Kruithof-de Julio M.
Curr Pharm Des. 2012;18(27):4103-13. PMID:2263008. Review.

8. KLF10 gene expression is associated with high fetal hemoglobin levels and with response to hydroxyurea treatment in β-hemoglobinopathy patients.
Borg J., Phylactides M., Bartsakoulia M., Tafrali C., Lederer C., Felice AE., Papachatzopoulou A., Kourakli A., Stavrou EF., Christou S., Hou J., **Karkampouna S.,** Lappa-Manakou C., Ozgur Z., van Ijcken W., von Lindern M., Grosveld FG., Georgitsi M., Kleanthous M., Philipsen S., Patrinos GP.
Pharmacogenomics. 2012 Oct;13(13):1487-500. PMID:23057549

9. Realities and expectations of pharmacogenomics and personalized medicine: impact of translating genetic knowledge into clinical practice.
Squassina A., Manchia M., Manolopoulos VG., Artac M., Lappa-Manakou C., **Karkampouna S**., Mitropoulos K., Del Zompo M., Patrinos GP.
Pharmacogenomics. 2010 Aug;11(8):1149-67. PMID:20712531. Review

## Patents

Kruithof-de Julio M., **Karkampouna S.,** Kruithof BPT., Goumans MJTH. Method for culturing tissue. INV-12MC349. WO/2014/173832. Filed April 17, 2014.
Published October 30, 2014.

## Acknowledgements

The work described in this thesis would not have been completed without the support of several people whom I would like to personally thank. Firstly, I am thankful to Prof.Dr. Peter ten Dijke for giving me the opportunity to be a member of the "TGFβ family", I appreciate all the time, advice and scientific discussions.
I would like to thank Dr. Marianna Kruithof-de Julio for her supervision throughout my PhD, for mentoring me to be critical and pragmatic and for transmitting her "out-of-the-box" way of thinking.
Many thanks to Midory, Maarten and all the former and current colleagues from the TGFβ group. To the fellow PhD students that I shared the office with, Yihao and Jie, special thanks and I am looking forward to both of your thesis.
I would like to thank all the people at the MCB department, in particular; Prof. Hans Tanke and the microscopy facility group; Annelies, Joop and Willem, Julia at the secretary office for all the help in the past months, all the members of Marie-Jose's group. Special thanks to Boudewijn Kruithof for scientific discussions. Thanks to all the S2-20 lab mates from the Parasitology group; Bruno, Gerard, Noemi. Ozge and Zary thanks for always being so positive and I wish you all the best. Maria Panagiotou, a.k.a Maraki, thank you and Kostas for the support, happy vibes, laughs and for sharing my Ioannina addiction :)
Many thanks to the colleagues at the Urology department and the neighboring D4 group of Endocrinology. From the department of Gastroenterology I would like to thank Minneke Coenraad, Hein Verspaget and Danny van der Helm for our collaboration during the past two years.
To my paranymphs; thank you both for your help and friendship!
To my wonderful friends; John B, Eva, Annemiek and Sven, Elvira, Elena, Maria K., Vasiliki, Aleksandra, Pavlos, Polynikis, Aristea and Stefanos, Nilhan, Irem, thank you all so much! Thanks to Cheryl and Nteleah, good luck with everything! To Stavros G. many thanks for the support, scientific discussions, the never-ending survival kits with superfood supplies and nerdy jokes. To my Argentinian niche Juan, Alejandro and Romina, thanks for the game nights and excruciating laughs. Romina thank you for all your help and great work with the artistic part and mostly your patience! Robert words are not enough; thank you for being there since day #1 and for your "do or do not, there is no try" attitude.
To my fantastic (nuclear and extended) big family; thank you all for your unconditional love and support !

*"Nothing behind me, everything ahead of me,*
*as is always so on the road".*

*Jack Kerouaq*
*On the road, 1957*