Hyaluronic acid inhibition by 4-methylumbelliferone reduces the expression of cancer stem cells markers during hepatocarcinogenesis

Caecilia H C Sukowati^{1,2*}, Beatrice Anfuso¹, Esteban Fiore³, Susan I Ie⁴, Alan Raseni⁵, Fulvia Vascotto⁵, Claudio Avellini⁶, Guillermo Mazzolini³, Claudio Tiribelli¹

- 1. Fondazione Italiana Fegato, AREA Science Park Basovizza, SS14 km 163.5, 34149 Trieste, Italy
- 2. Department of Medicine. University of Udine. Piazzale M. Kolbe 1, 33100 Udine, Italy
- Gene Therapy Laboratory, Facultad de Ciencias Biomédicas, Universidad Austral, Avenida Presidente Perón 1500, B1629ODT Derqui-Pilar, Buenos Aires, Argentina
- Laboratory of Hepatitis and Emerging Diseases, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, 10310 Jakarta, Indonesia
- Institute for Maternal and Child Health Institute for Research and Health Care Burlo Garofolo, Via dell'Istria, 65, 34137 Trieste, Italy
- Department of Medical and Biological Sciences, University Hospital Santa Maria della Misericordia, Piazzale Santa Maria della Misericordia 15, 33100 Udine, Italy

*Correspondence and requests for materials should be addressed to CHCS <u>caecilia.sukowati@fegato.it</u>

Supplementary Figure

Fig. S1. Unprocessed original scans of Western blot in Fig 5B.

CD133



Actin

ACTIN	A2066	1:1000				20	15.09	.29	
1	1	2	3	4	٢	6	7 8	9	
1	- 1					_			
		L]				J
	-			, -			-	- 14	
400		0 mM		(0.5m	М.	2.01	nu	







Fig. S3. The staining of HA-coated plate by Alcianblue 8GX showing the positivity of HA.



SREP-18-26478A_Ms – supplementary material

Supplementary Table

Table S1. List of primers used in this study

Gene	Primer F $(5' \rightarrow 3')$	Primer R $(5' \rightarrow 3')$	Ref
Human			
18sr-RNA	TAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG	1
ACTB	CGCCGCCAGCTCACCATG	CACGATGGAGGGGAAGACGG	This study
EpCAM	GAATAATAATCGTCAATGCCAGTG	CGCTCTCATCGCAGTCAG	This study
CD90/THY-1	AGAGACTTGGATGAGGAG	CTGAGAATGCTGGAGATG	This study
CD133/Prominin 1	CATCTGCTCTCTGCTGAC	AACTTAATCCAACTCCAACC	This study
CD44	AGGAAGAAGGATGGATATGGACTC	TTACTCTGCTGCGTTGTCATTG	This study
HAS1	TACAACCAGAAGTTCCTGGG	CTGGAGGTGTACTTGGTAGC	2
HAS2	GTGGATTATGTACAGGTTTGTGA	TCCAACCATGGGATCTTCTT	2
HAS3	GAGATGTCCAGATCCTCAACAA	CCCACTAATACACTGCACAC	2
HYAL1	GATGTCAGTGTCTTCGATGTGGTA	GGGAGCTATAGAAAATTGTCATGTCA	3
HYAL2	CTAATGAGGGTTTTGTGAACCAGAATAT	GCAGAATCGAAGCGTGGATAC	3
CDH1	GGAACTATGAAAAGTGGGCTTG	AAATTGCCAGGCTCAATGAC	4
CDH2	GACGGTTCGCCATCCAGAC	TCGATTGGTTTGACCACGG	5
VIM	AACTTCTCAGCATCACGATGAC	TTGTAGGAGTGTCGGTTGTTAAG	This study
BCL2a	GTGTGTGGAGAGCGTCAAC	CGGTTCAGGTACTCAGTCATC	This study

BAX	TCGCCCTTTTCTACTTTG	CCCATGATGGTTCTGATC	This study
PUMA	CCTGTAAGATACTGTATATGC	CCACTGTTCCAATCTGAT	This study
Mouse			
Gapdh	CCAGTATGACTCCACTCACG	CTCGCTCCTGGAAGATGGTG	6
Actb	AATAAGTGGTTACAGGAAGTC	ATGAAGTATTAAGGCGGAAG	7
Has1	TTCCACTGTGTGTCCTGCAT	TGTACCAGGCCTCCAAGAAC	8
Has2	GGGACCTGGTGAGACAGAAG	ATGAGGCAGGGTCAAGCATA	8
Has3	TCCCCAAGTAGGAGGTGTTG	TTGCACACAGCCAAAGTAGG	8
Hyal1	CCGTAATGCCCTACGTCCAGA	GCCTGGCATGATTCCTTGGT	8
Hyal2	AGCCGCAACTTTGTCAGTTT	GAGTCCTCGGGTGTATGTGG	8
Cd44	CTCCTGAAGAAGACTGTA	CACGGTTGACAATAGTTAT	This study
Cd90	AACTTCACCACCAAGGAT	TTGTCTCTATACACACTGATACT	8
Epcam	ATTGTGGTGGTGTCATTAG	TCCTTTATCTCAGCCTTCT	This study
Fsp1	CAGAAGGTGATGAGCAACT	AGGACAGGAAGACACAGTA	This study
Acta2	GGCATCAATCACTTCAAC	TCTGGTCACCTGTATGTA	This study
CD133/Prominin 1	GACATCTCAGTTGATTCCAAGG	CATGGCGCATTCTGCTTCTGC	9

References:

1. Schmittgen, T. D. & Zakrajsek, B. A. Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative

RT-PCR. J. Biochem. Biophys. Methods 46, 69–81 (2000).

- Twarock, S. *et al.* Synthesis of hyaluronan in oesophageal cancer cells is uncoupled from the prostaglandin-cAMP pathway. *Br. J. Pharmacol.* 157, 234–243 (2009).
- 3. Li, L., Asteriou, T., Bernert, B., Heldin, C. H. & Heldin, P. Growth factor regulation of hyaluronan synthesis and degradation in human dermal fibroblasts: importance of hyaluronan for the mitogenic response of PDGF-BB. *Biochem. J.* **404**, 327–336 (2007).
- 4. Lin, C. W. *et al.* Epithelial cell adhesion molecule regulates tumor initiation and tumorigenesis via activating reprogramming factors and epithelial-mesenchymal transition gene expression in colon cancer. *J. Biol. Chem.* **287**, 39449–39459 (2012).
- 5. Kroepil, F. et al. Down-regulation of CDH1 is associated with expression of SNAI1 in colorectal adenomas. PloS One 7, e46665 (2012).
- 6. Hayashi, A., Suzuki, H., Itoh, K., Yamamoto, M. & Sugiyama, Y. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochem. Biophys. Res. Commun.* **310**, 824–829 (2003).
- 7. Raab, R. M., Bullen, J., Kelleher, J., Mantzoros, C. & Stephanopoulos, G. Regulation of mouse hepatic genes in response to diet induced obesity, insulin resistance and fasting induced weight reduction. *Nutr. Metab.* **2**, 15 (2005).
- 8. Hunt, L. C. *et al.* Hyaluronan synthesis and myogenesis: a requirement for hyaluronan synthesis during myogenic differentiation independent of pericellular matrix formation. *J. Biol. Chem.* **288**, 13006–13021 (2013).
- 9. Kemper, K., Tol, M. J. P. M. & Medema, J. P. Mouse tissues express multiple splice variants of prominin-1. PloS One 5, e12325 (2010).