Single cell sequencing

Karel Smetana ml.[1,2], Lukáš Lacina [1,2,3], Michal Kolář [2,4]

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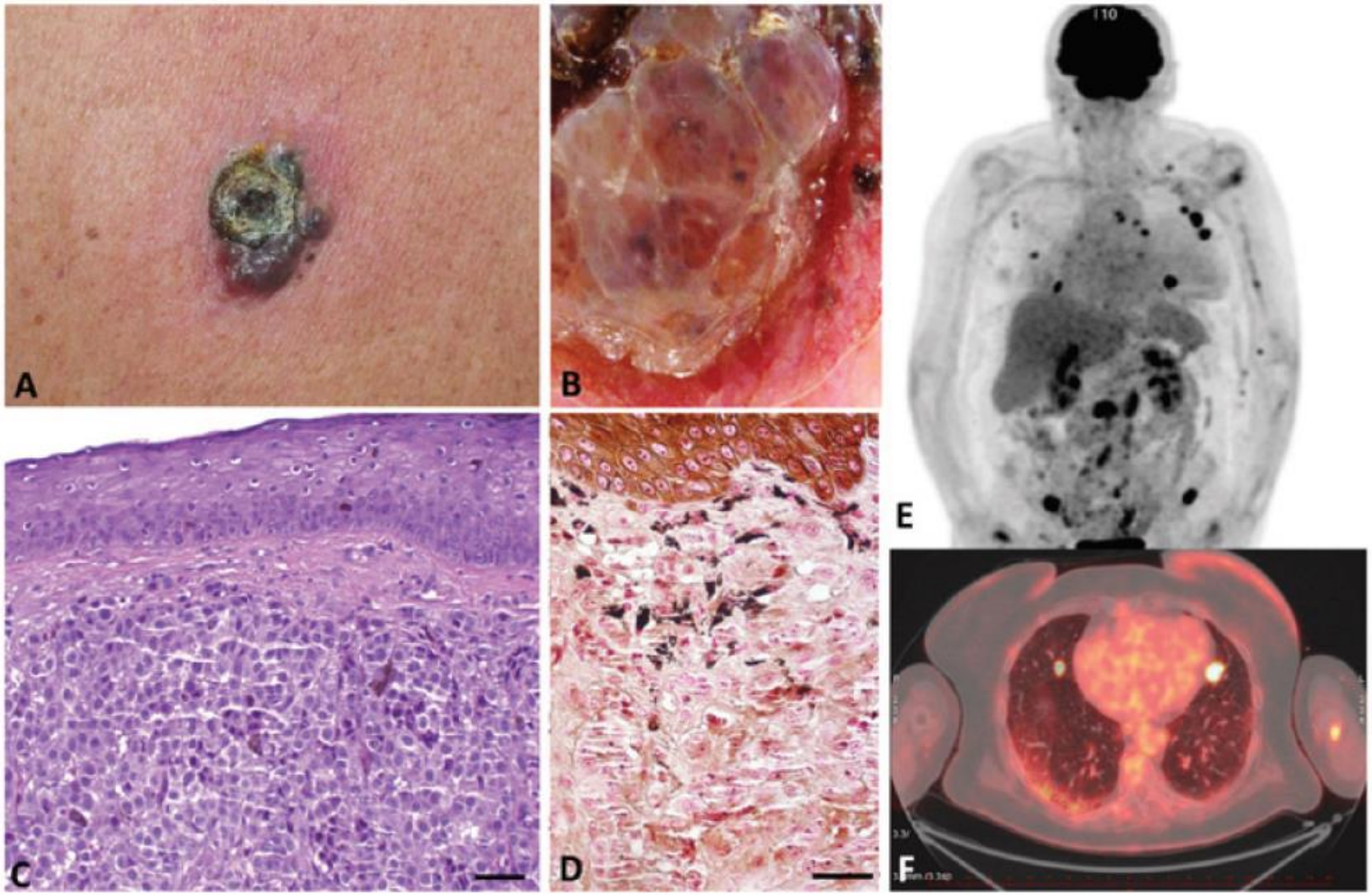
INTERNATIONAL JOURNAL OF ONCOLOGY 57: 619-630, 2020

Cutaneous melanoma dissemination is dependent on the malignant cell properties and factors of intercellular crosstalk in the cancer microenvironment (Review)

ONDŘEJ KODET[1-3], JAN KUČERA[1,2], KAROLÍNA STRNADOVÁ[1,3], BARBORA DVOŘÁNKOVÁ[1,3], JIŘÍ ŠTORK[2], LUKÁŠ LACINA[1-3] and KAREL SMETANA Jr[1,3]



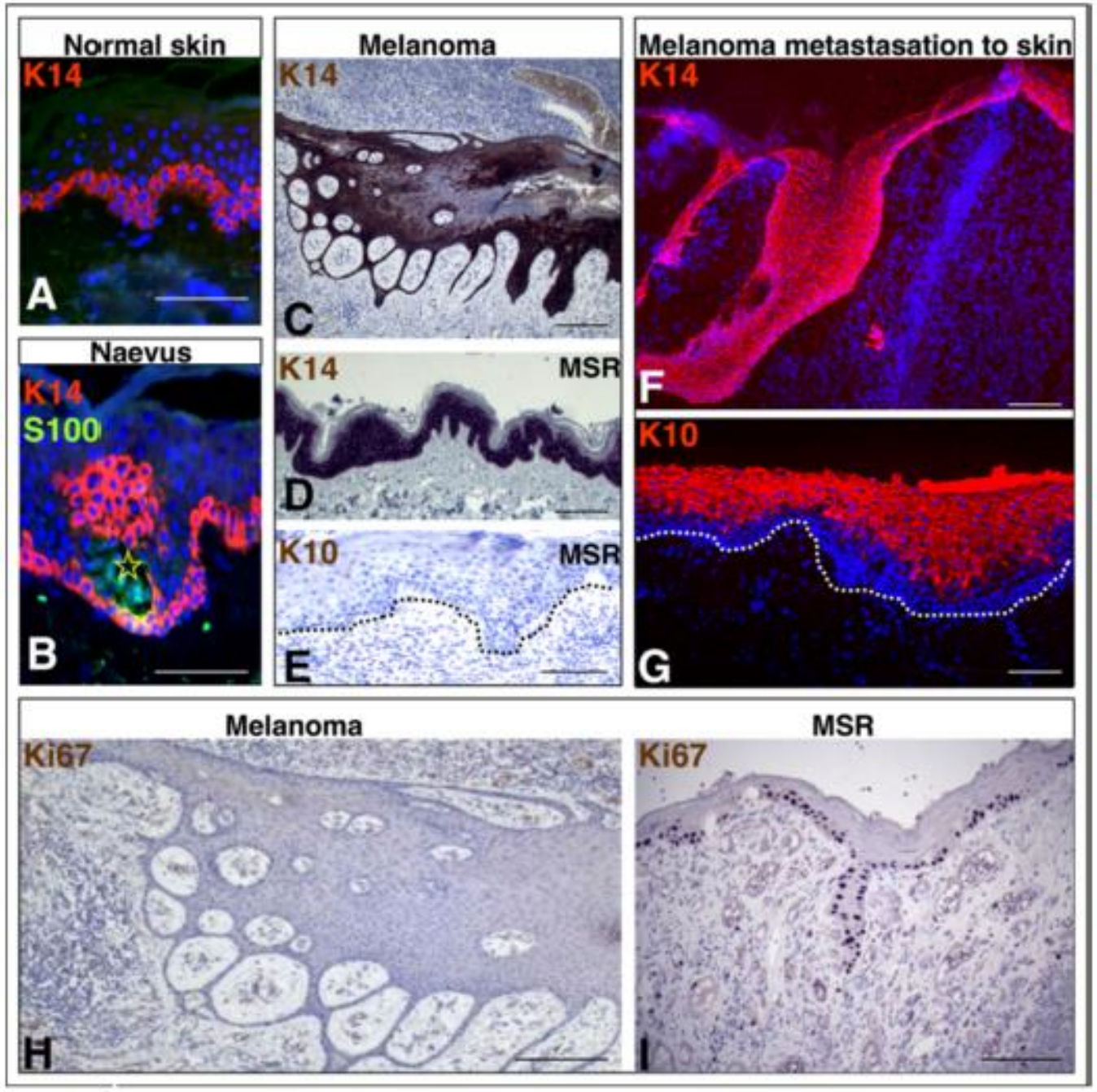
cancers

Editorial

Targeted Therapies for Melanoma

Karel Smetana, Jr. [1,2,*], Lukáš Lacina [1,2,3] and Ondřej Kodet [1,2,3]

Even, a very small CMM can generalize. The therapy of advanced stage is problematic, even when targeted/biological therapy is employed.



Kodet *et al. Molecular Cancer* 2015, **14**:1
http://www.molecular-cancer.com/content/14/1/1

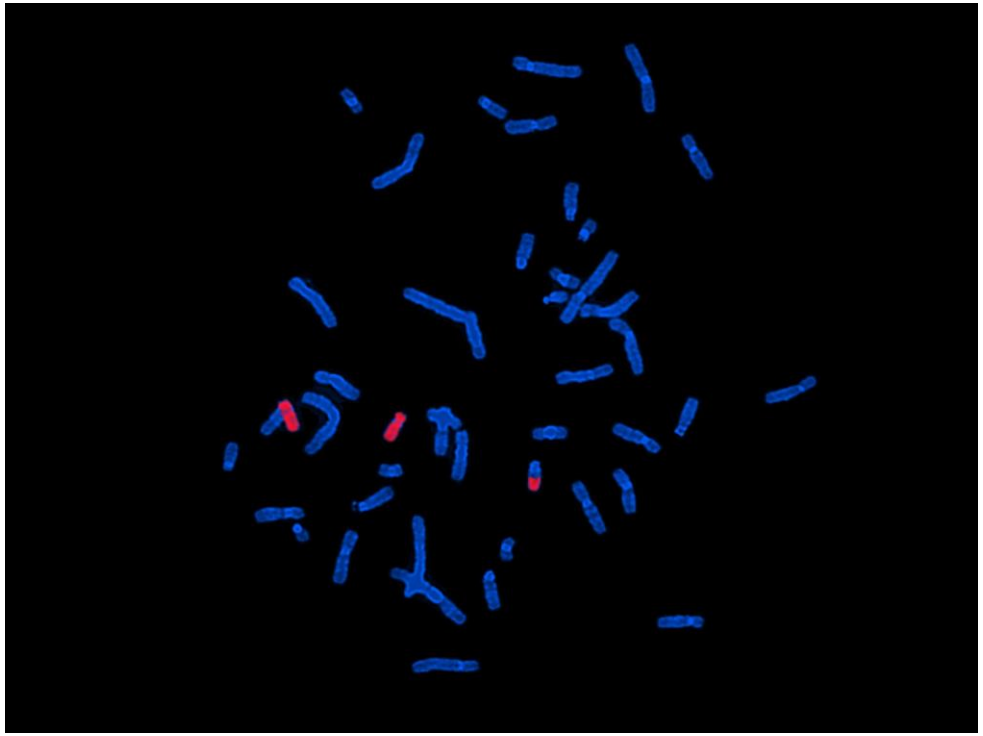
MOLECULAR CANCER

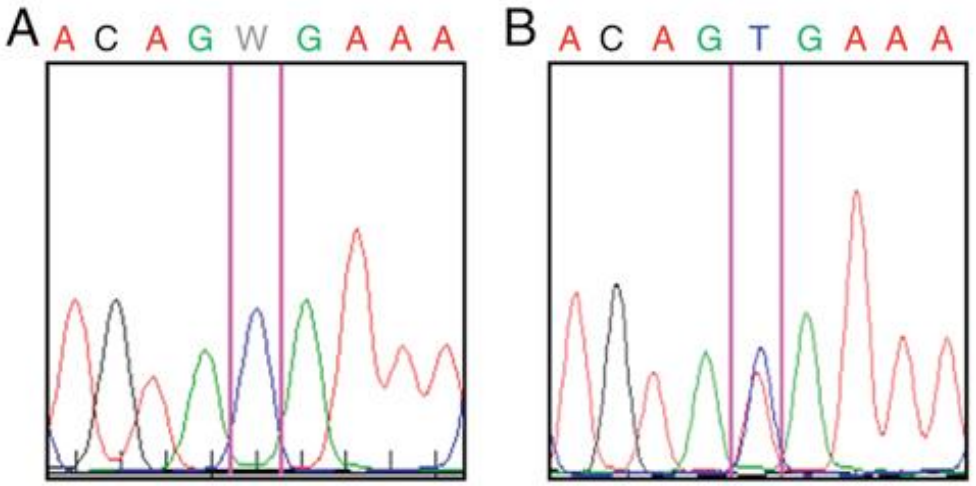
RESEARCH Open Access

Melanoma cells influence the differentiation pattern of human epidermal keratinocytes

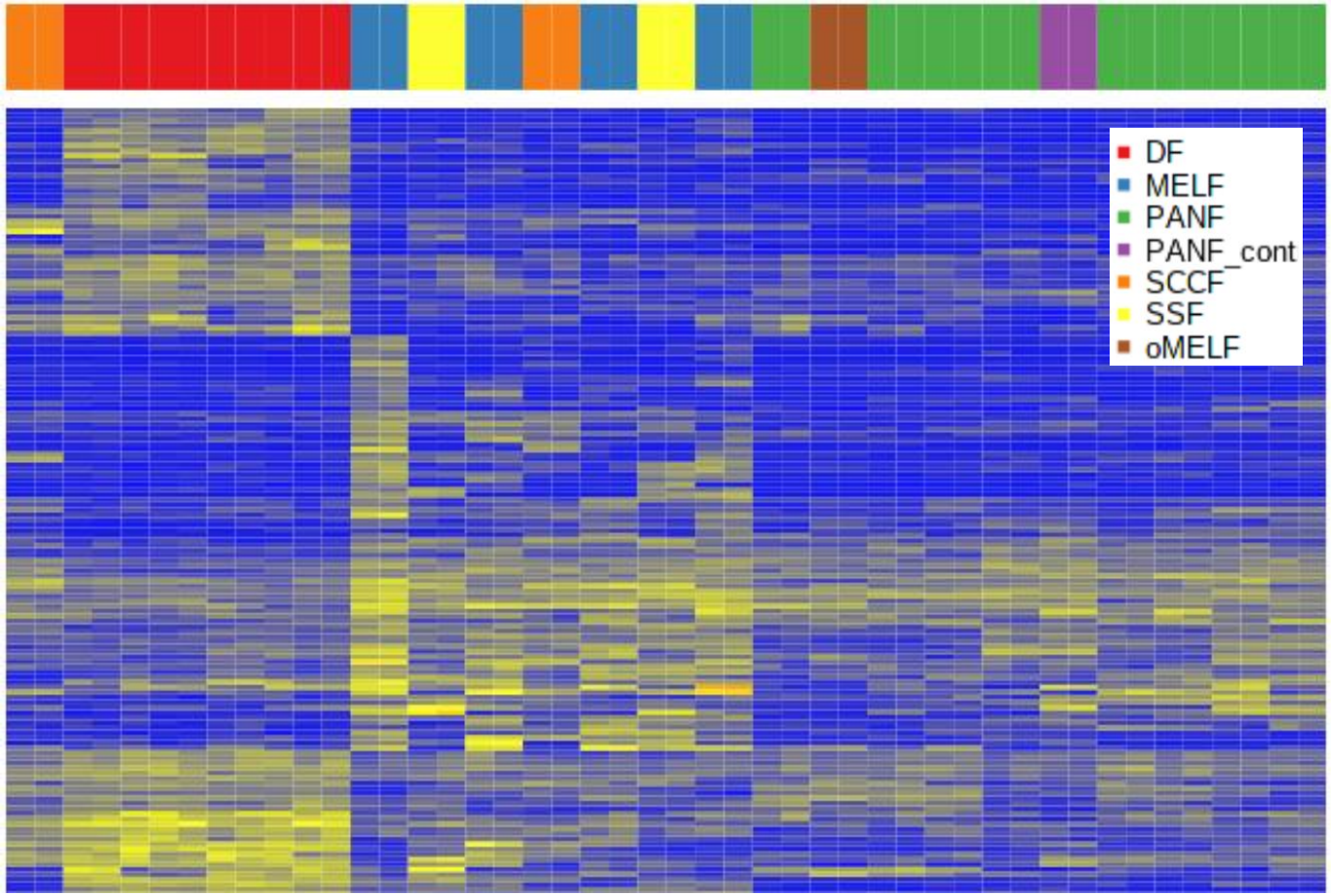
Ondřej Kodet[1,2], Lukáš Lacina[1,2,3], Eliška Krejčí[1], Barbora Dvořánková[1], Miloš Grim[1], Jiří Štork[2], Daniela Kodetová[4], Čestmír Vlček[5], Jana Šáchová[5], Michal Kolář[5], Hynek Strnad[5*] and Karel Smetana[1*]

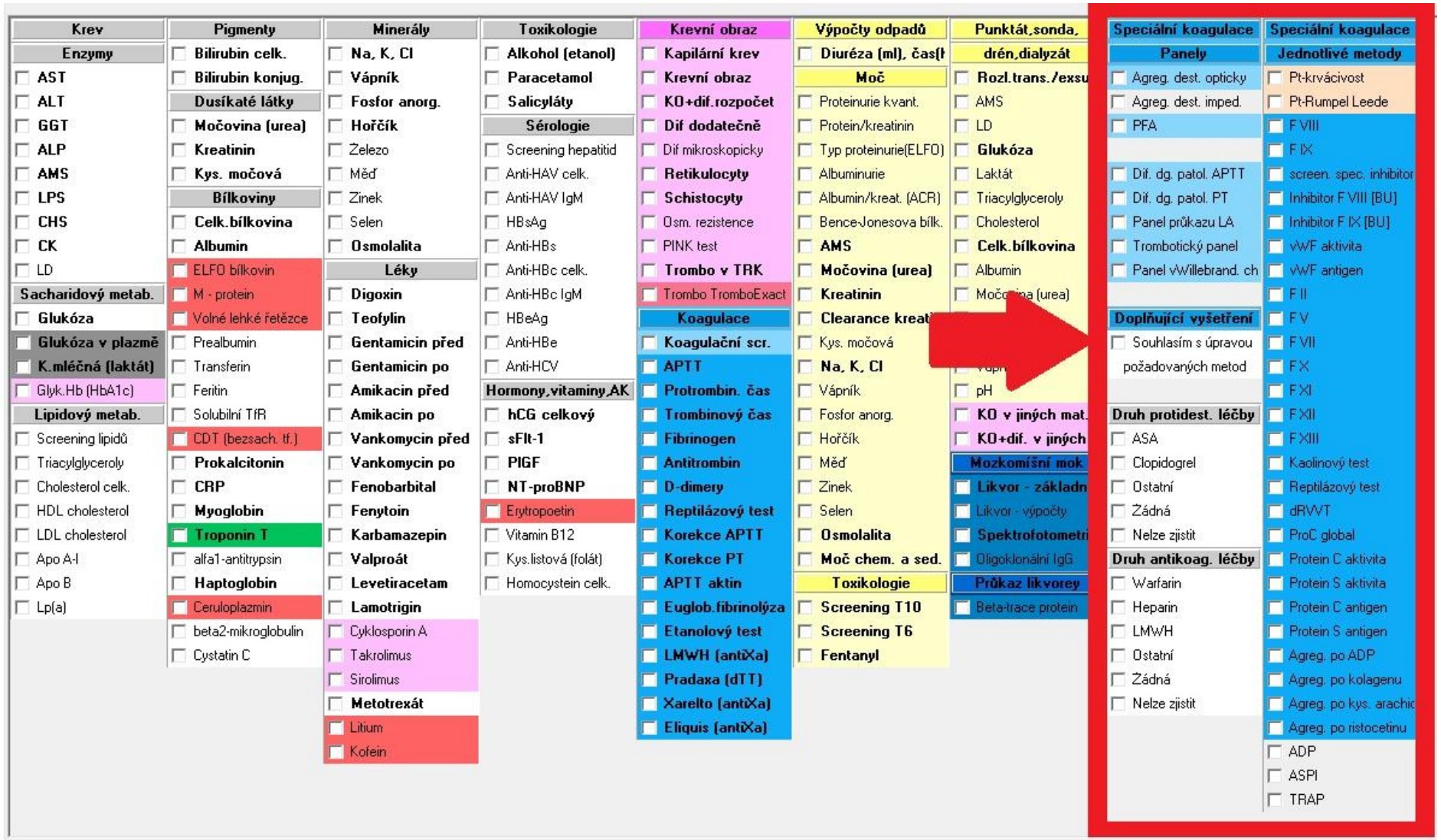
Morphological/immunohistochemical investigation is basis for tumour diagnostic. Unfortunately, inspection of multiple markers at single cell level is limited.



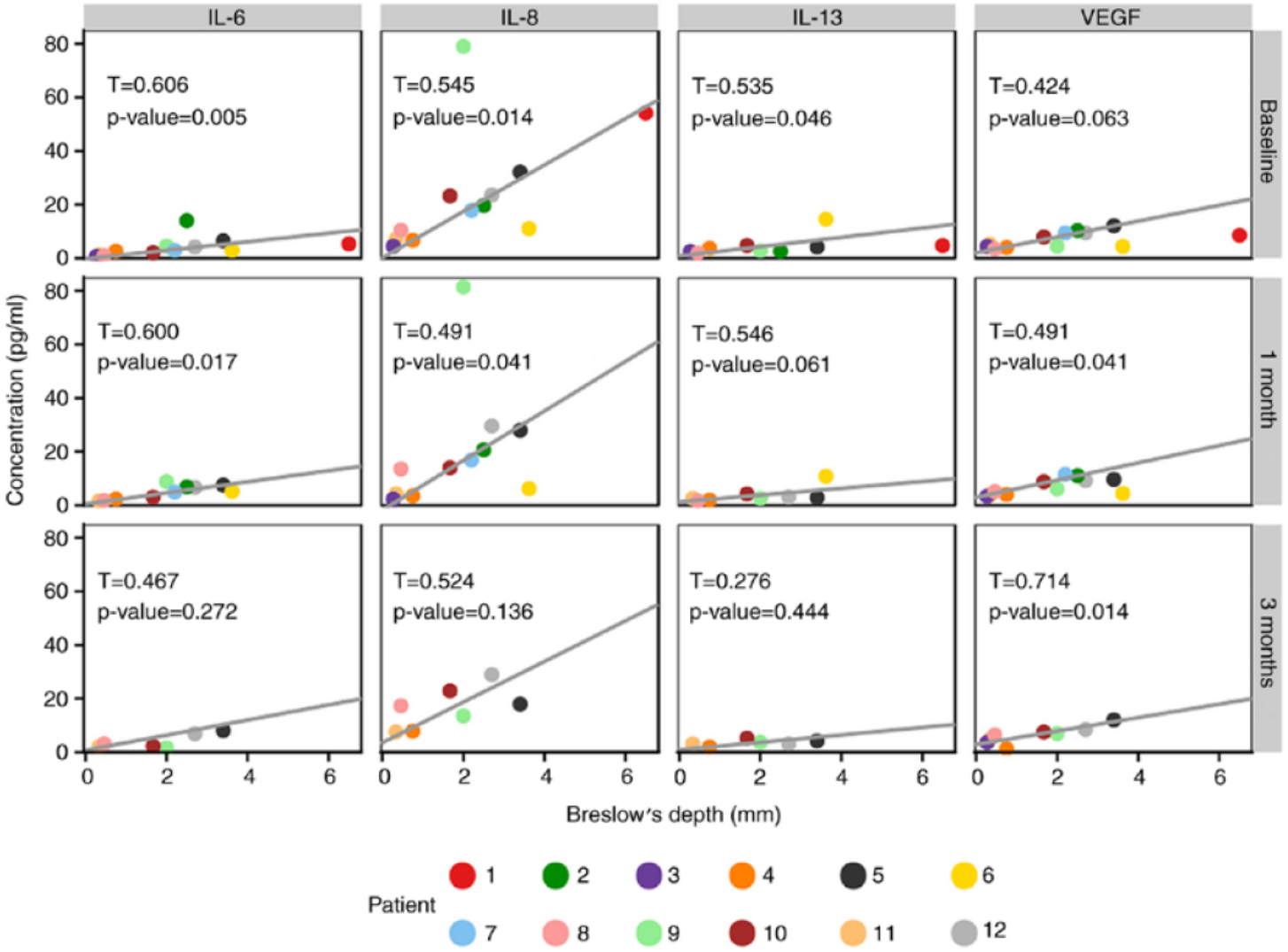


Mutation analysis and can be performed by FISH or in isolated DNA. Similarly, the transcriptome can be evaluated from isolated mRNA.





Biochemical investigation is essential for evaluation of patient status but it cannot reflect the situation of individual cell population.



ONCOLOGY REPORTS 42: 1793-1804, 2019

Serum proteomic analysis of melanoma patients with immunohistochemical profiling of primary melanomas and cultured cells: Pilot study

JAN KUČERA[1,2*], KAROLÍNA STRNADOVÁ[2,3*], BARBORA DVOŘÁNKOVÁ[2,3], LUKÁŠ LACINA[1-3], IVANA KRAJSOVÁ[1], JIŘÍ ŠTORK[1], HANA KOVÁŘOVÁ[4], HELENA KUPCOVÁ SKALNÍKOVÁ[4], PETR VODIČKA[4], JAN MOTLÍK[4], PAVEL DUNDR[5], KAREL SMETANA Jr[2,3] and ONDŘEJ KODET[1-3]

Level of inflammation-supporting markers in serum of patients with CMM reflects the Breslow´s depth in mm.

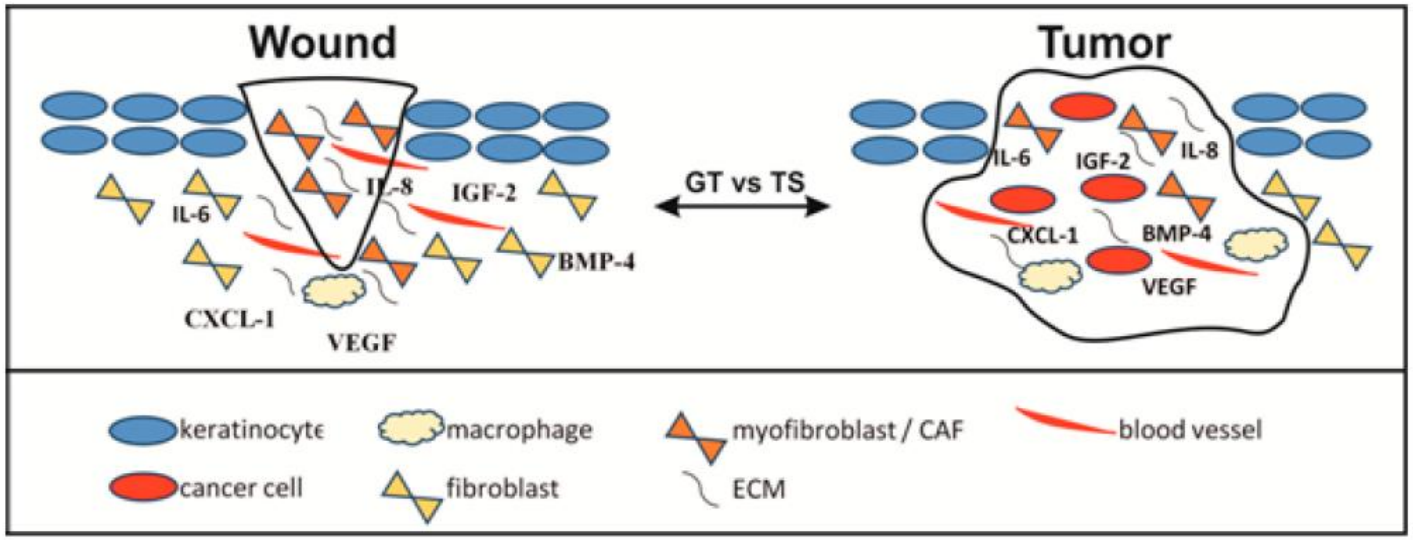
molecules

MDPI

Review

How Signaling Molecules Regulate Tumor Microenvironment: Parallels to Wound Repair

Peter Gál [1,2,3,*], Lenka Varinská [1,2], Lenka Fáber [2], Štepán Novák [4,5], Pavol Szabo [1,4,6], Petra Mitrengová [3], Andrej Mirossay [2], Pavel Mučaji [3,*] and Karel Smetana Jr. [4,6,*]



Tumour represents a complex ecosystem composed from cancer cells, cancer-associated fibroblasts, inflammatory cells and their products. The multiparametric analysis of distinct cells is necessary for personalized tailor-made therapy of patient.

Histochemistry and Cell Biology (2018) 149:503–516
https://doi.org/10.1007/s00418-018-1650-4

ORIGINAL PAPER

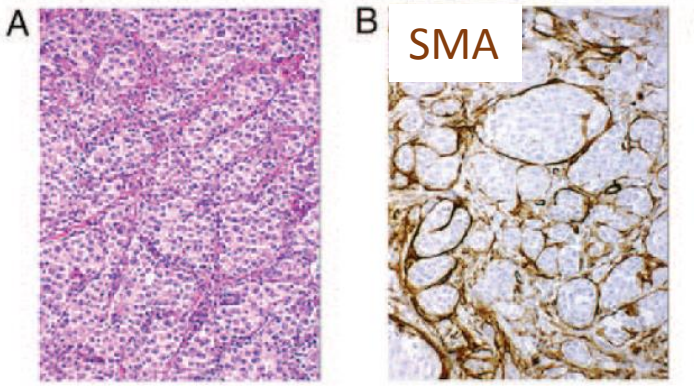
Fibroblasts potentiate melanoma cells in vitro invasiveness induced by UV-irradiated keratinocytes

Njainday Pulo Jobe[1,2,8] · Veronika Živicová[3,4] · Alžběta Mifková[3,4] · Daniel Rösel[1,2] · Barbora Dvořánková[2,3] · Ondřej Kodet[2,3,5] · Hynek Strnad[6] · Michal Kolář[6] · Aleksi Šedo[7] · Karel Smetana Jr.[2,3] · Karolina Strnadová[2,3] · Jan Brábek[1,2] · Lukáš Lacina[2,3,5]

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 41: 2687-2703, 2018

Microenvironment-driven resistance to B-Raf inhibition in a melanoma patient is accompanied by broad changes of gene methylation and expression in distal fibroblasts

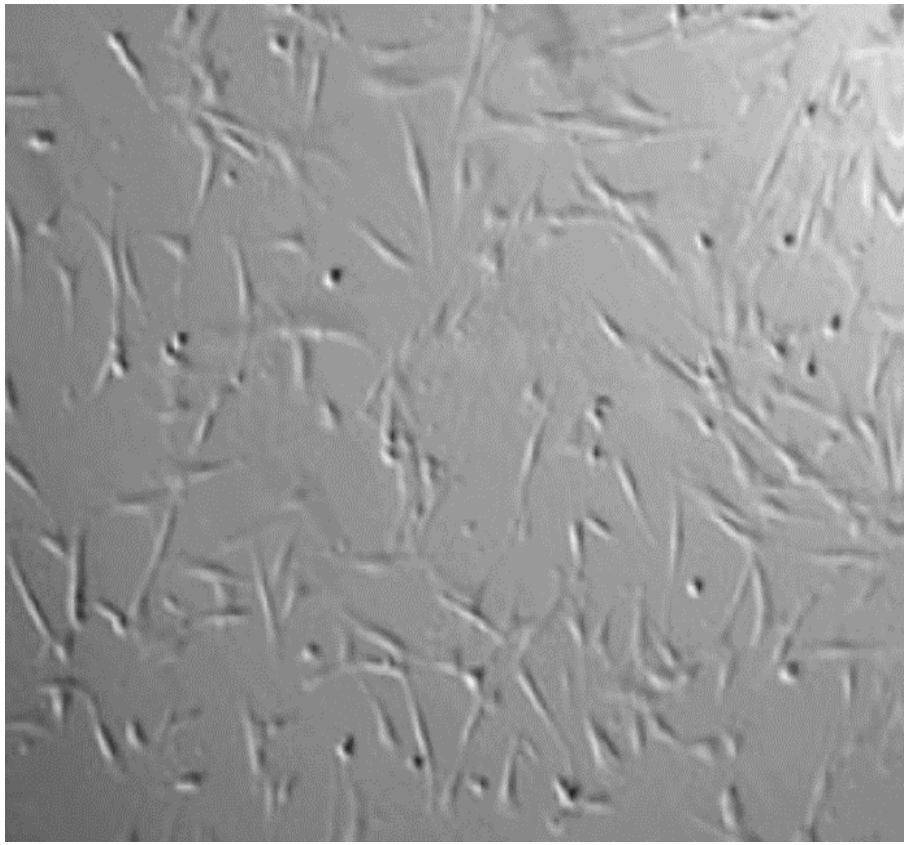
ONDŘEJ KODET[1-4], BARBORA DVOŘÁNKOVÁ[1,3], BĚLA BENDLOVÁ[5], VLASTA SÝKOROVÁ[5], IVANA KRAJSOVÁ[4], JIŘÍ ŠTORK[2,4], JAN KUČERA[1,2,4], PAVOL SZABO[1,3], HYNEK STRNAD[6], MICHAL KOLÁŘ[6], ČESTMÍR VLČEK[6], KAREL SMETANA Jr[1,3] and LUKÁŠ LACINA[1-4]

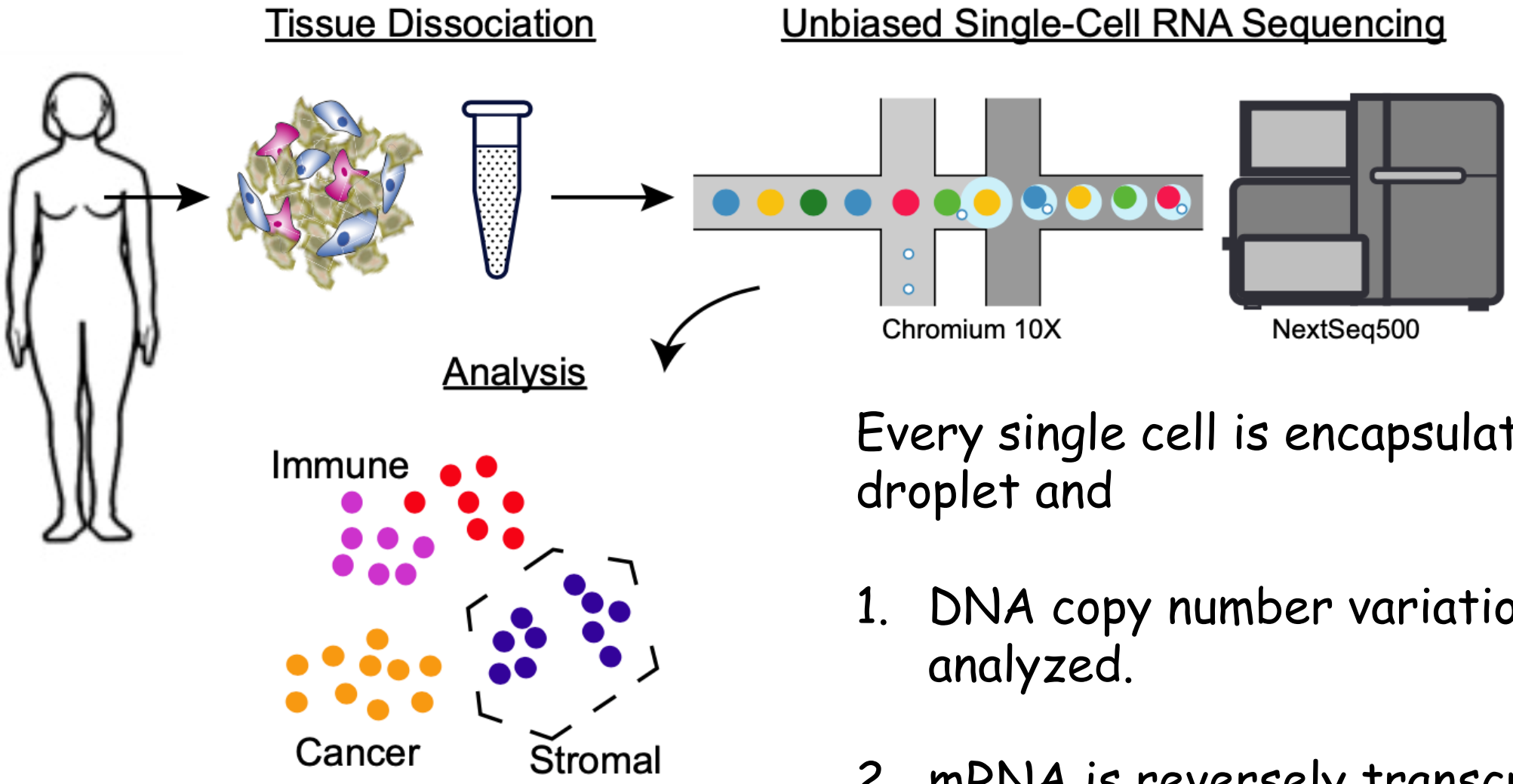


Symbol	Gene name	LFC	FDR	Kerat	Melanoma
IL8	Interleukin 8	5.5	2.6e−06	+	+
ACAN	Aggrecan	4.27	3.6e−07	+	+
IL6	Interleukin 6 (interferon, beta 2)	3.87	7.5e−13	+	+
IL1B	Interleukin 1, beta	2.52	3.6e−08		
CXCL1	Chemokine (C–C–C motif) ligand 1 (melanoma growth stimulating activity, alpha)	2.49	0.0045	+	+
HBEGF	Heparin-binding EGF-like growth factor	1.74	8.5e−06	+	
BDNF	Brain-derived neurotrophic factor	1.46	5.3e−06		+
TGFB2	Transforming growth factor, beta 2	1.23	0.00017		+
IGFBP7	Insulin-like growth factor binding protein 7	1.23	0.00012	+	
GAP43	Growth associated protein 43	1.21	2.3e−05	+	
CXCL16	Chemokine (C–X–C motif) ligand 16	1.17	8.3e−05		+
BMP6	Bone morphogenetic protein 6	1.11	0.0032		
KAZALD1	Kazal-type serine peptidase inhibitor domain 1	1.03	0.016		
VEGFC	Vascular endothelial growth factor C	1.02	0.0066	+	+
CTGF	Connective tissue growth factor	0.95	0.022		+
PDGFRL	Platelet-derived growth factor receptor-like	0.93	0.055		+
LEPREL1	Leprecan-like 1	0.91	0.017		
IL17D	Interleukin 17D	0.88	0.00021		+
VEGFA	Vascular endothelial growth factor A	0.82	0.029	+	+
BMP2	Bone morphogenetic protein 2	0.73	0.028		+
LEPRE1	Leucine proline-enriched proteoglycan (leprecan) 1	0.72	0.0011		

CAF produce many factors supporting inflammation.

Cells isolated from tumour are morphologically almost identical. However, their expression profile will be multicolored.





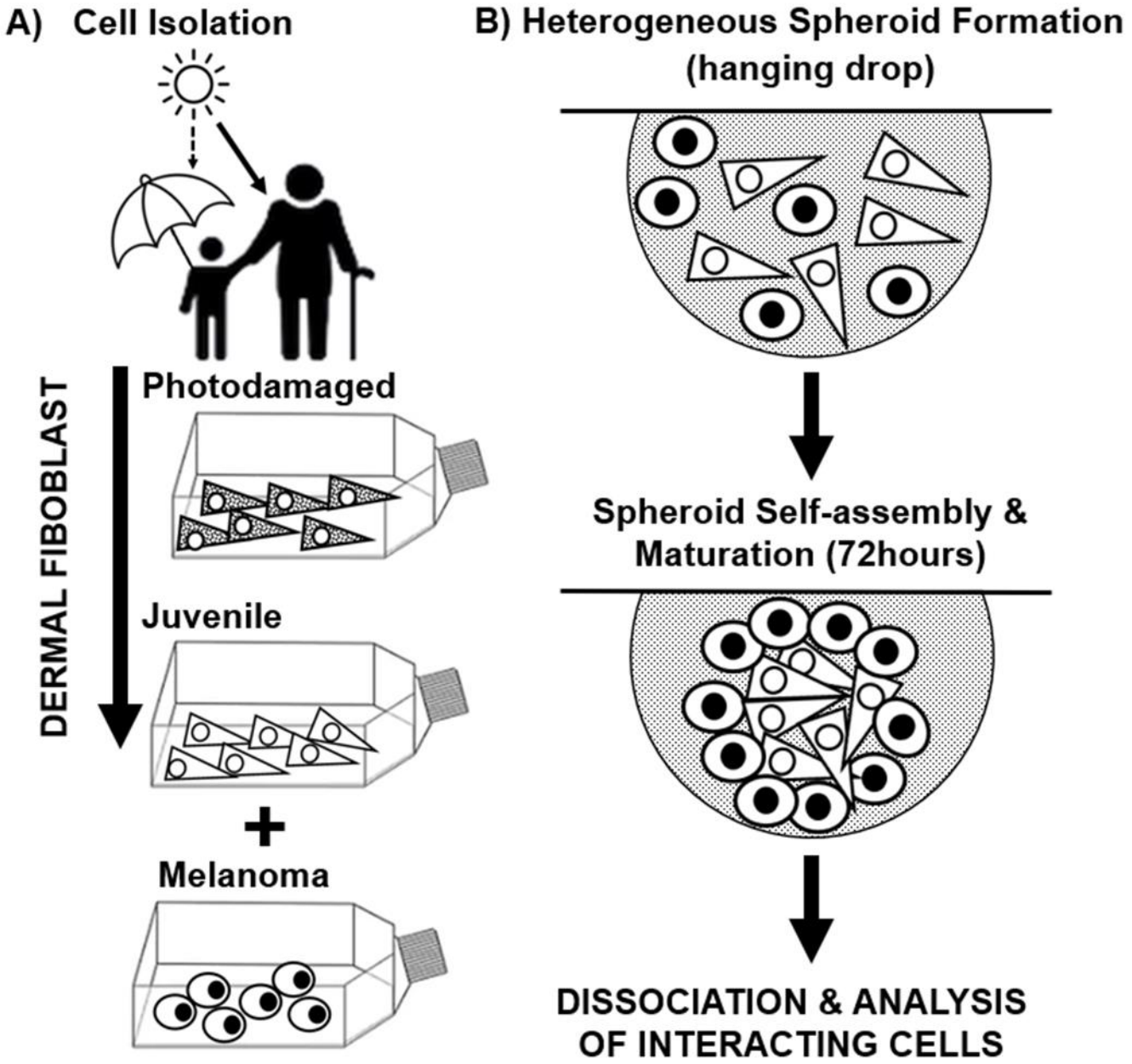
Every single cell is encapsulated in a lipid droplet and

1. DNA copy number variation can be analyzed.

2. mRNA is reversely transcribed to DNA, amplified and analyzed.

Bioinformatic analysis is an indispensable part of the experiment.

Adapted from Wu et al, EMBO J (2020)



cancers

MDPI

Article

Single-Cell RNA Sequencing Unravels Heterogeneity of the Stromal Niche in Cutaneous Melanoma Heterogeneous Spheroids

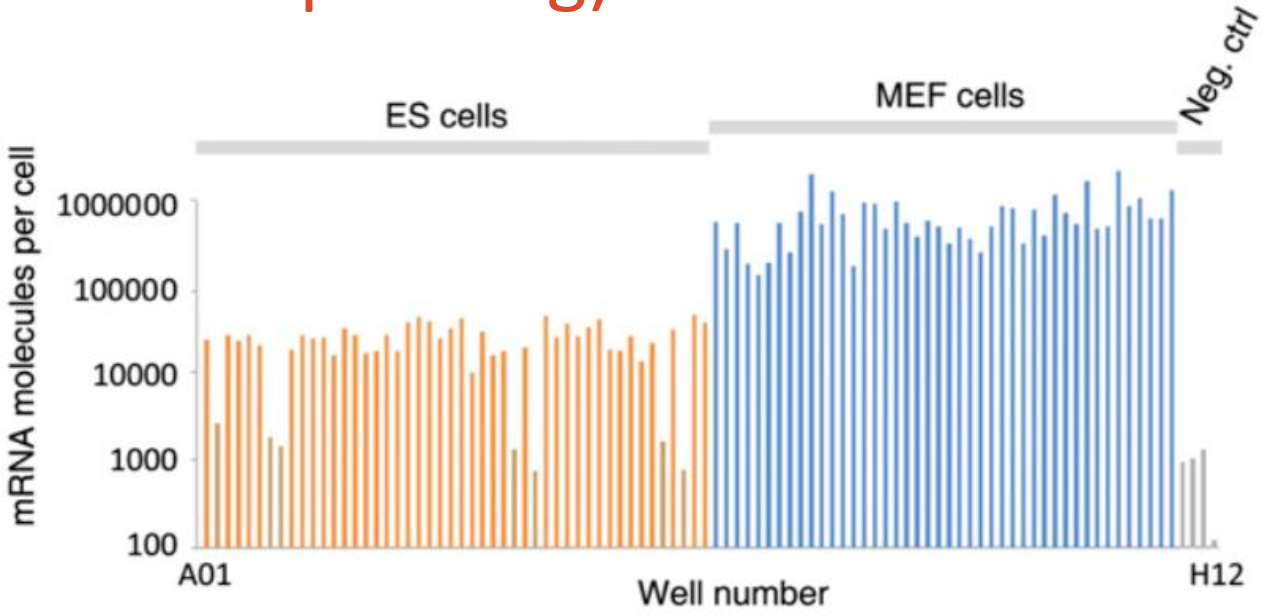
Jiří Novotný [1,2,†], Karolína Strnadová [3,4,†], Barbora Dvořánková [3,4], Šárka Kocourková [1], Radek Jakša [5], Pavel Dundr [5], Václav Pačes [1], Karel Smetana Jr [3,4], Michal Kolář [1,*] and Lukáš Lacina [3,4,6,*]

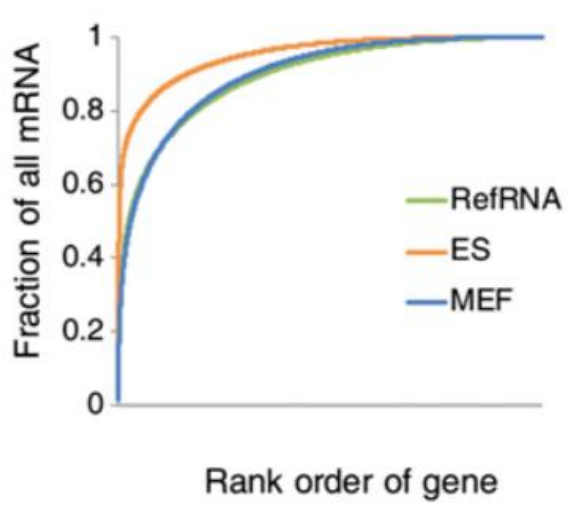
Cancers **2020**, *12*, 3324; doi:10.3390/cancers12113324

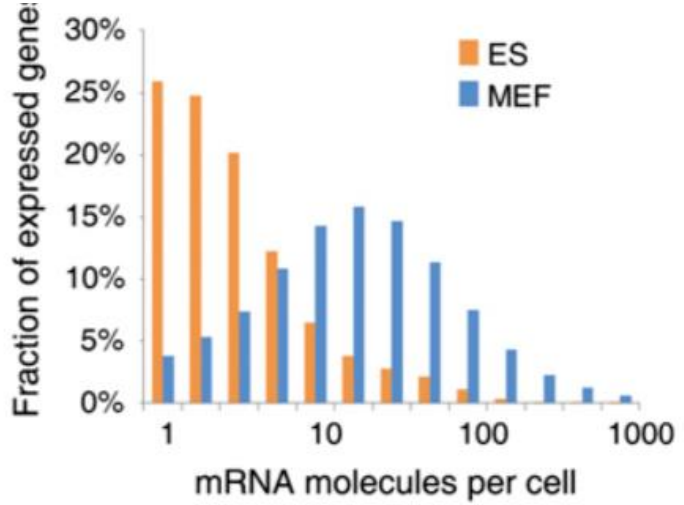
We prepared fibroblasts from the UV-exposed adult (PDF) and UV-protected juvenile (JDF) skin. These fibroblasts and CMM cells (G361) were used for construction of heterogeneous spheroids that were analyzed by procedure of single cell sequencing.

Typical setup (3’ mRNA profiling)

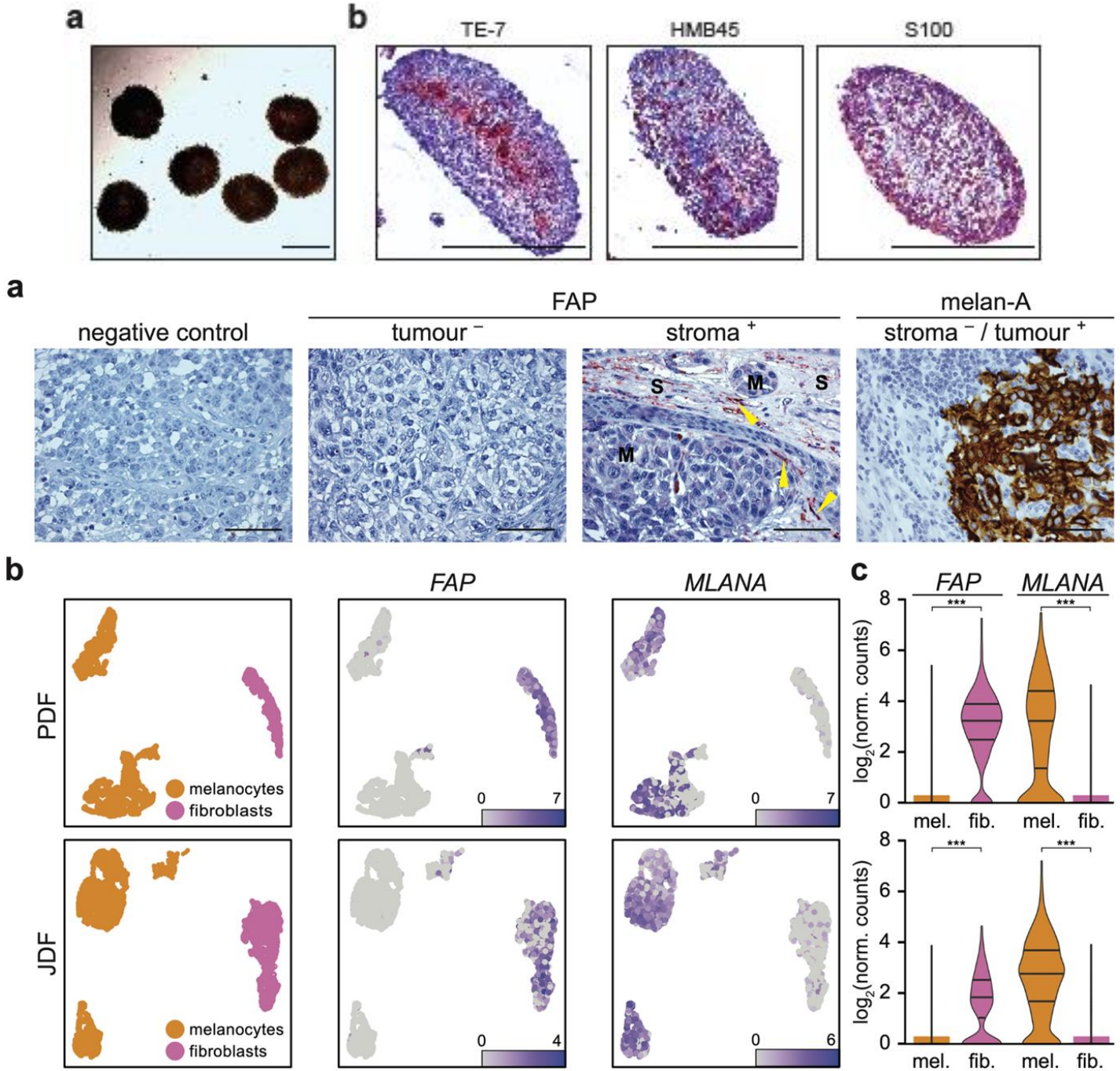
- Library prep: target 4 thousand cells per sample
- Sequencing: at least 25 thousand reads per cell
- Cost: expect high ten thousands CZK per sample





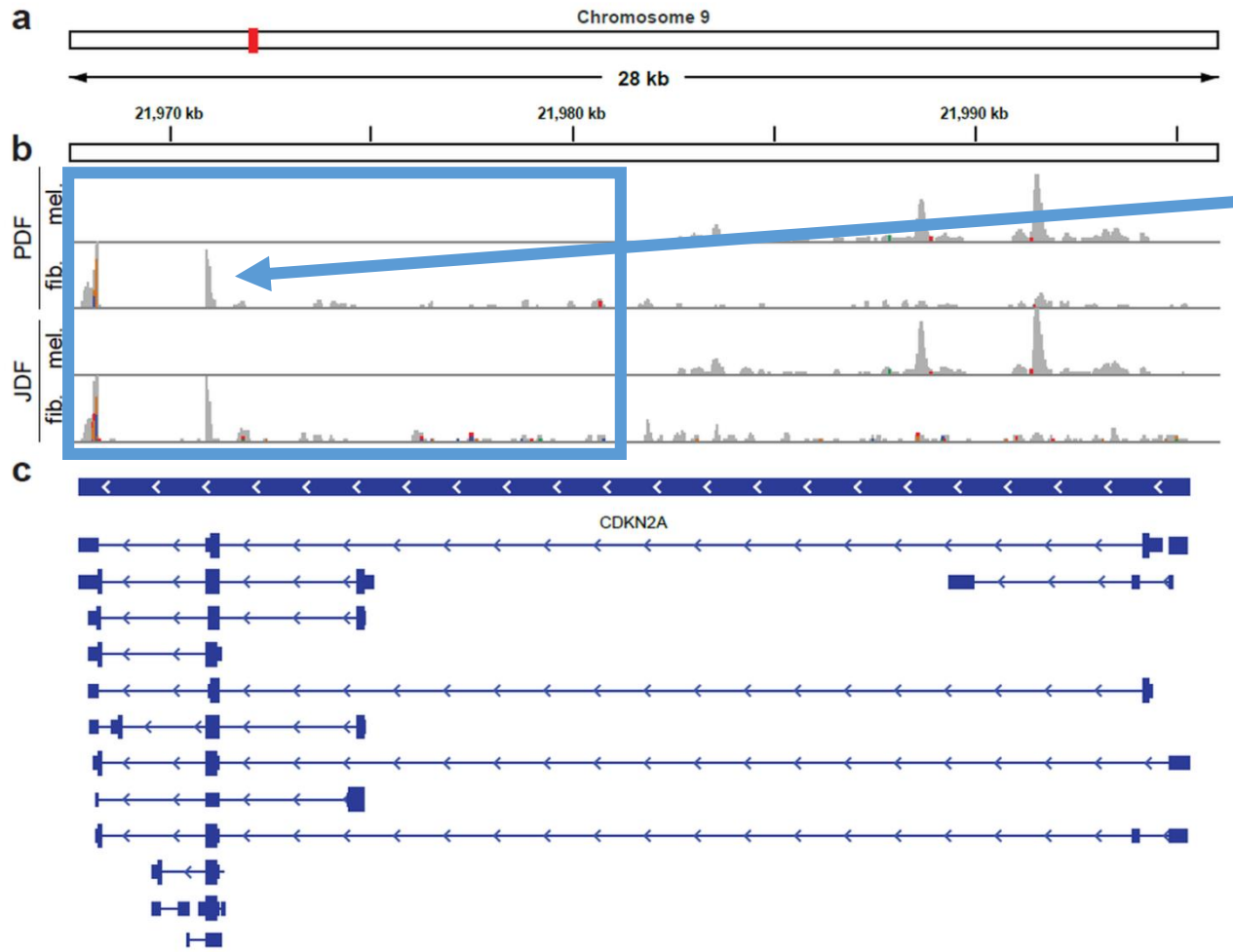


Islam et al. Genome Research 2011

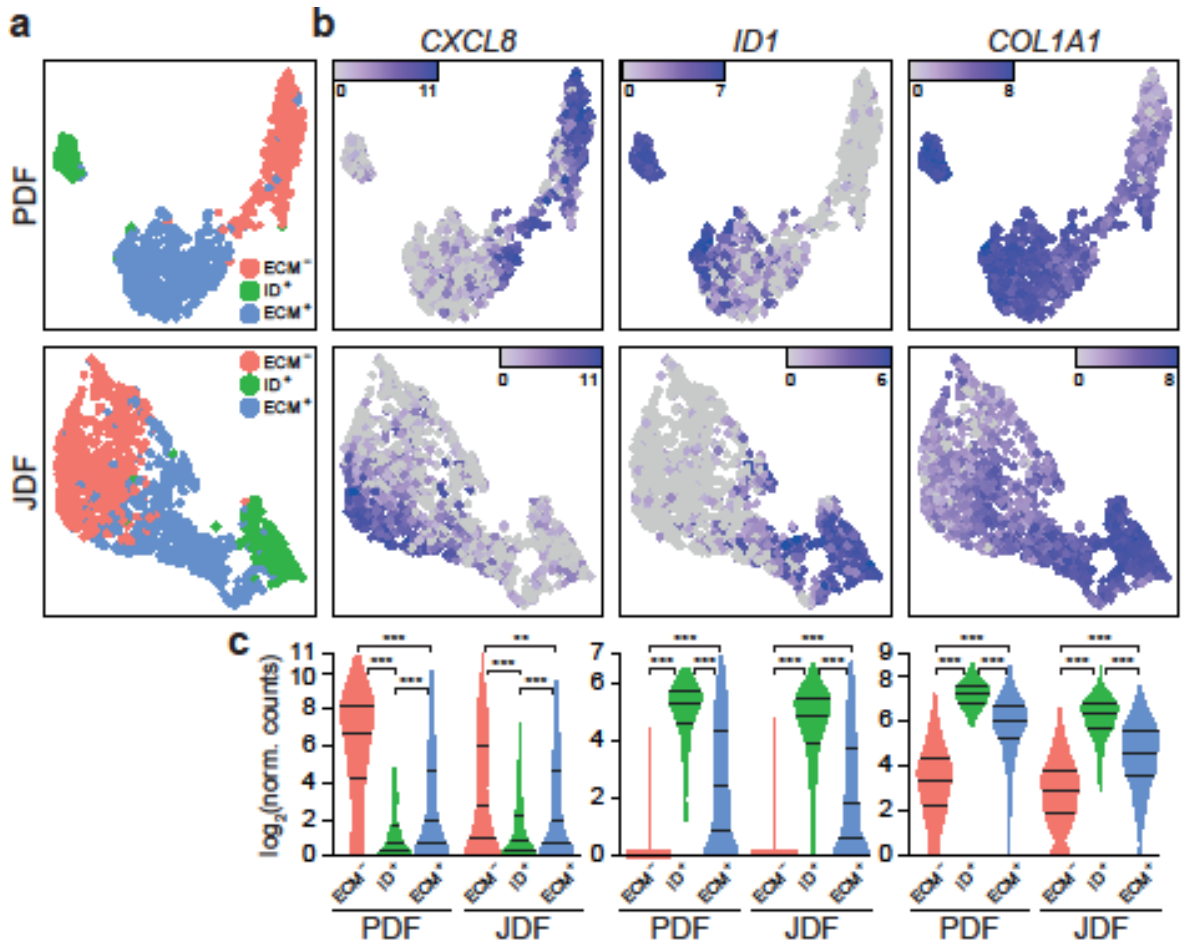


The heterogeneous spheres containing melanoma cells (G361), adult (PDF) or juvenile (JDF) fibroblasts were prepared. The cell types are diagnosed according to expression of melanoma gene (*MLANA*) and CAF gene (*FAP*) by scRNA-seq analysis.

Supplementary Figure S3

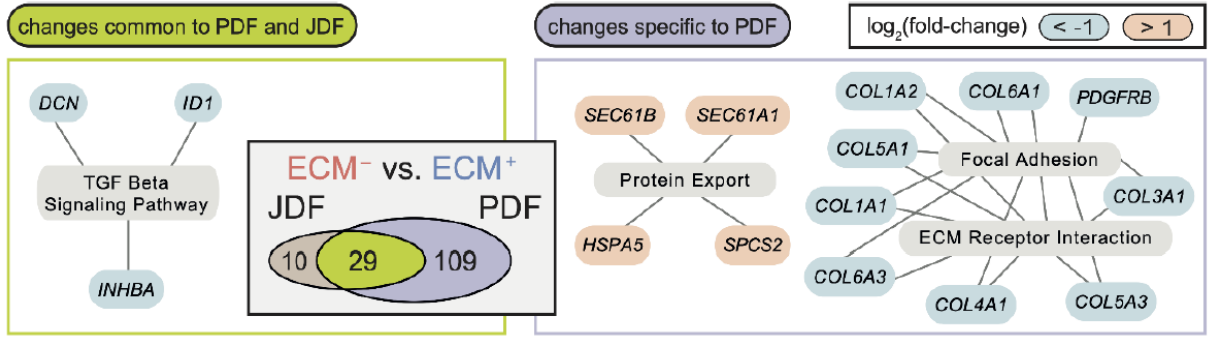


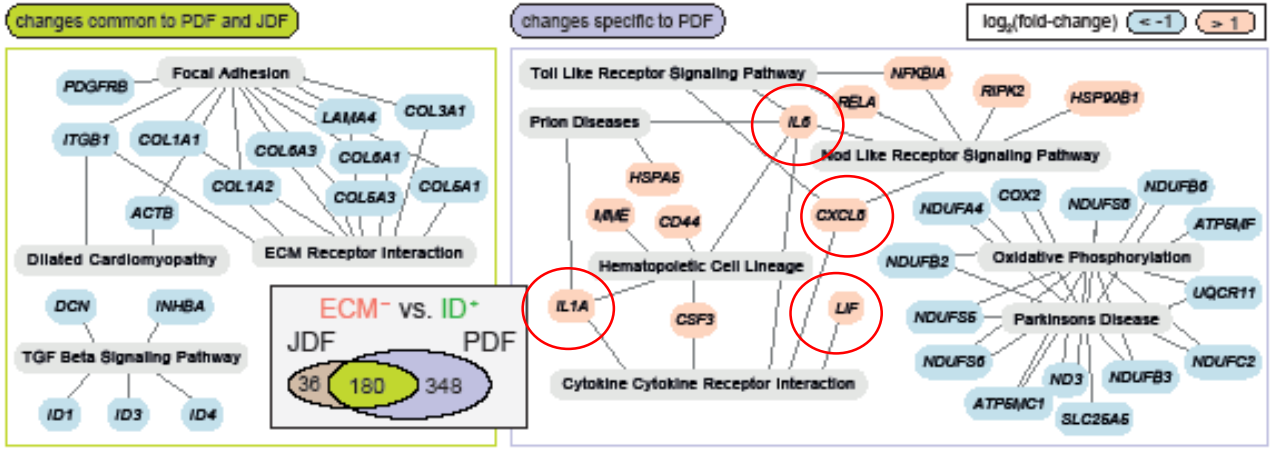
G361 CMM cells have defect of *CDKN2A* gene that is normal in both types of fibroblasts

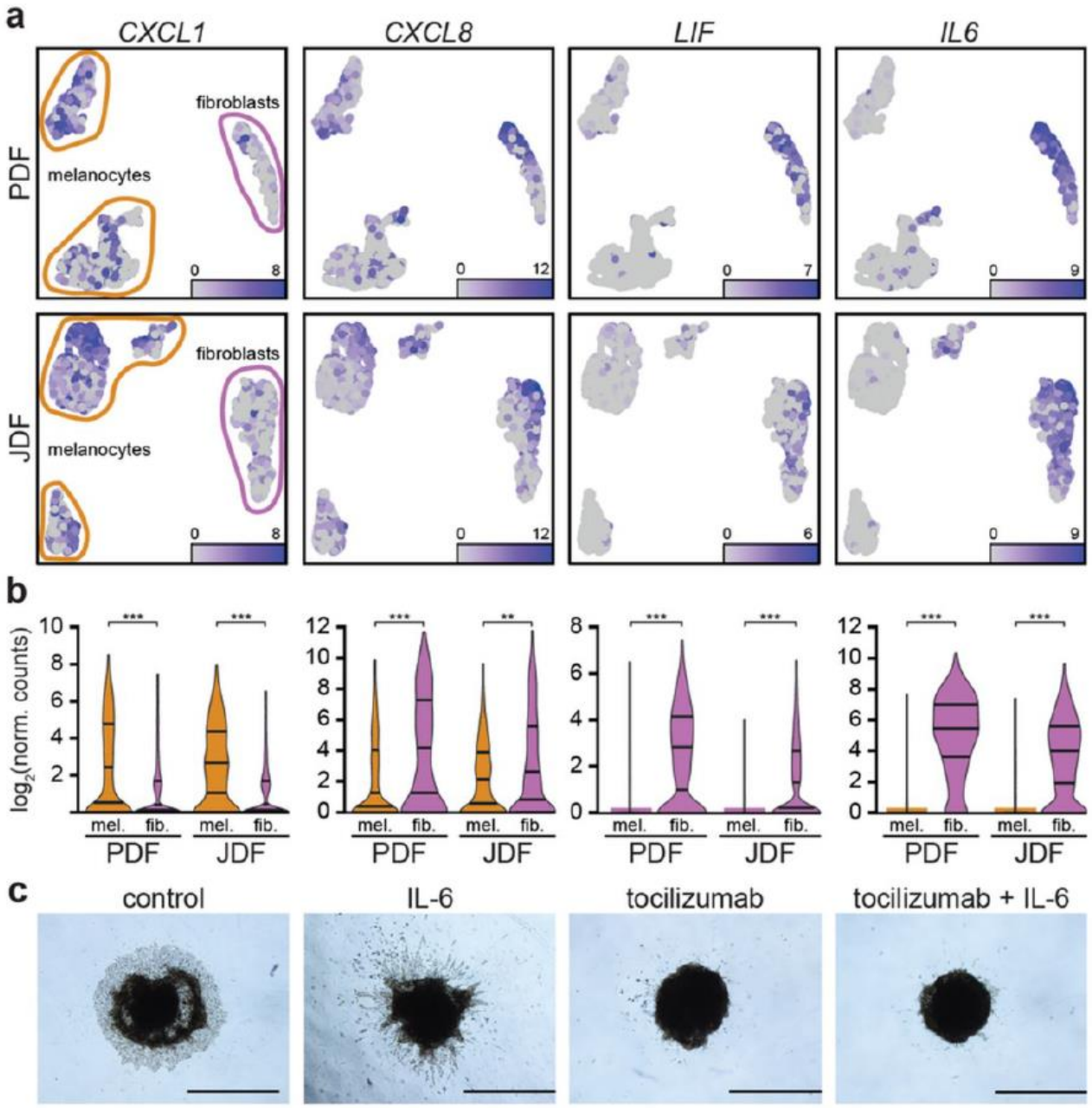


Fibroblasts from adult UV-exposed donors exhibit lower activity of genes encoding ECM and mitochondrial genes. On the other hand, the activity of genes encoding inflammation-supporting factors is higher than in juvenile fibroblasts.

Both types of fibroblasts in spheres form heterogeneous population of cells that differ by activity of genes encoding ECM (*COL1A1*), or inflammation supporting factors (*CXCL8*) and *ID* genes depending on TGF-β signalization.



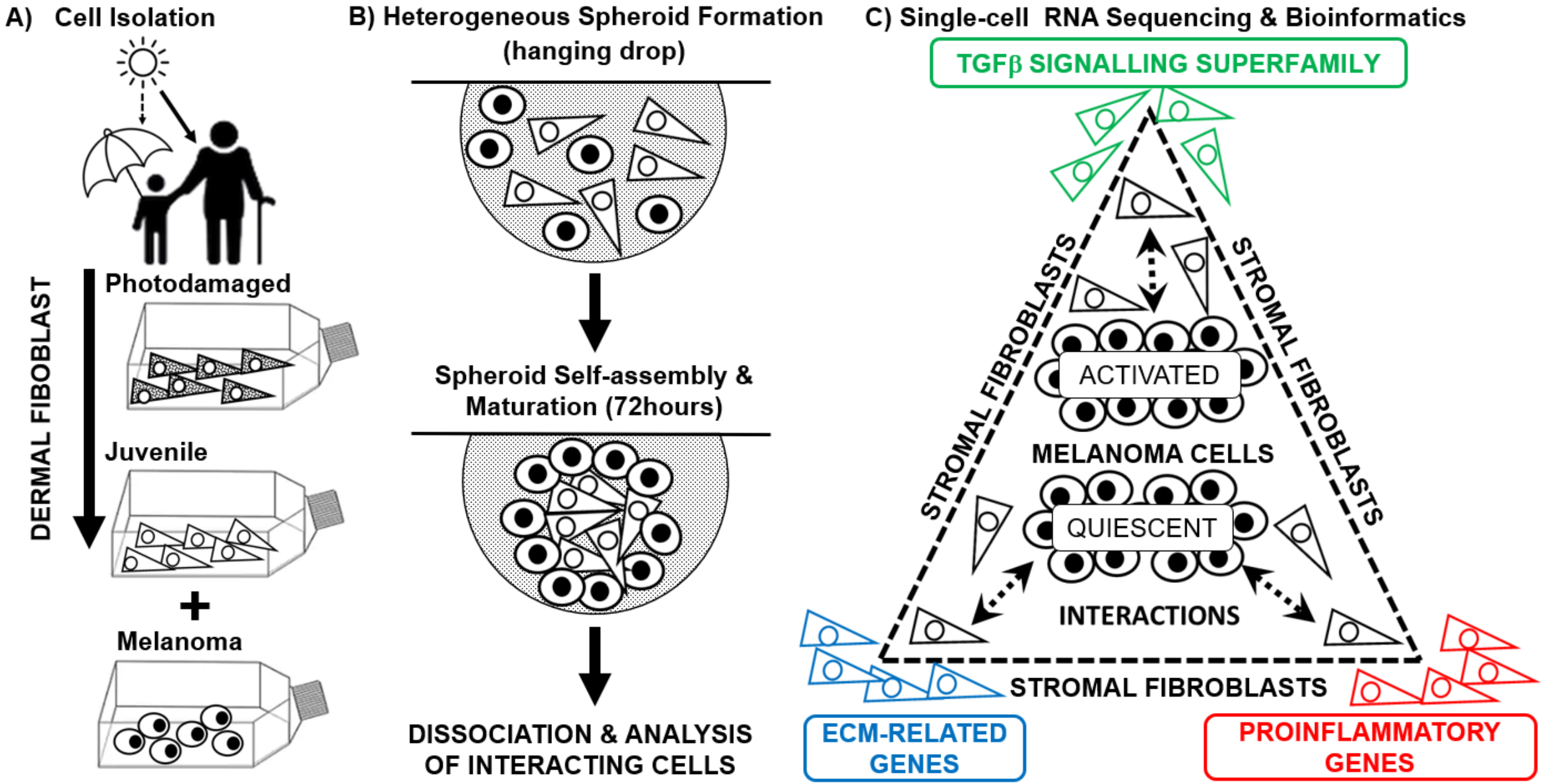




While gene for chemokine *CXCL1* is more expressed by melanoma cells, gene for chemokine *CXCL8* is expressed by both fibroblasts and melanoma cells. Genes for *LIF* and *IL6* are strongly expressed by fibroblasts.

Humanized anti-IL-6R antibody Tocilizumab inhibits migration of melanoma cells in 3D collagen gel.

Heterogeneity of the Stromal Fibroblast in Cutaneous Melanoma Spheroids



scRNA-seq represents an unique robust tool to evaluate the genetic profile of each cell such as mapping of mutations and transcription profile at single cell level and the whole genome scale. This procedure is suitable for experimental purpose and in future also for clinical diagnostics.

Karlova Universita, 1. lékařská fakulta, Praha
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Ústav patologie: P.Dundr, R.Jakša

Ústav molekulární genetiky AVČR, Praha: Š.Kocourková, J.Novotný, V.Pačes

Finanční podpora:

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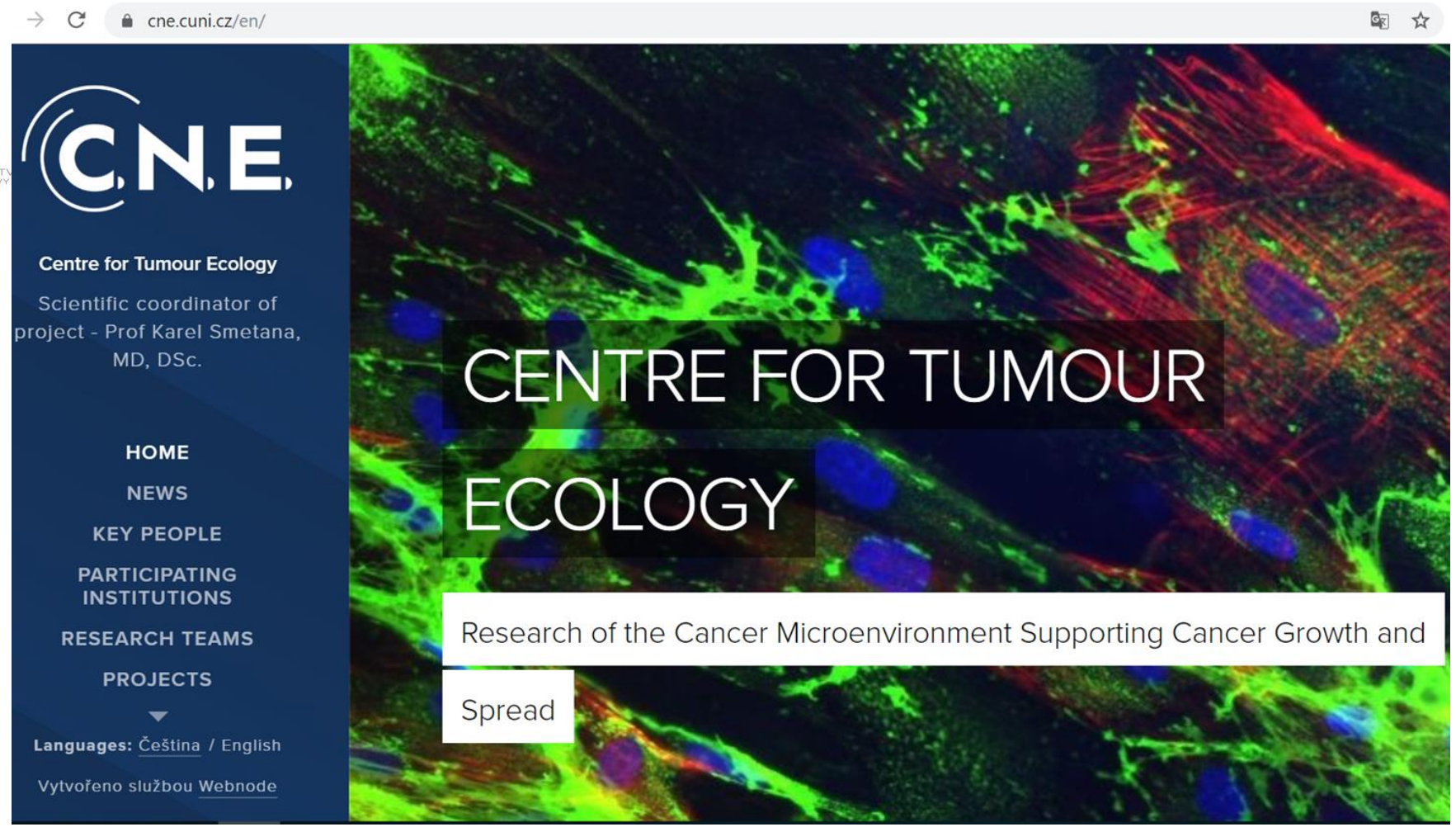
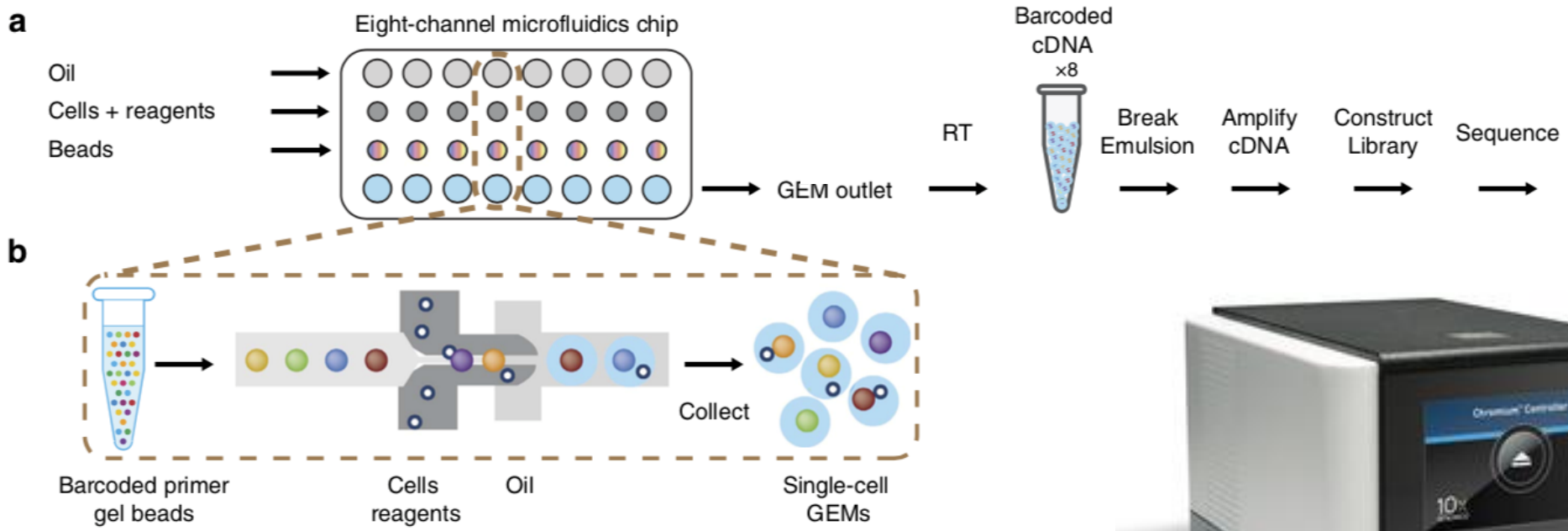
GAČR
GRANTOVÁ AGENTURA ČESKÉ REPUBLIKY

MINISTERSTVO ZDRAVOTNICTVÍ
ČESKÉ REPUBLIKY

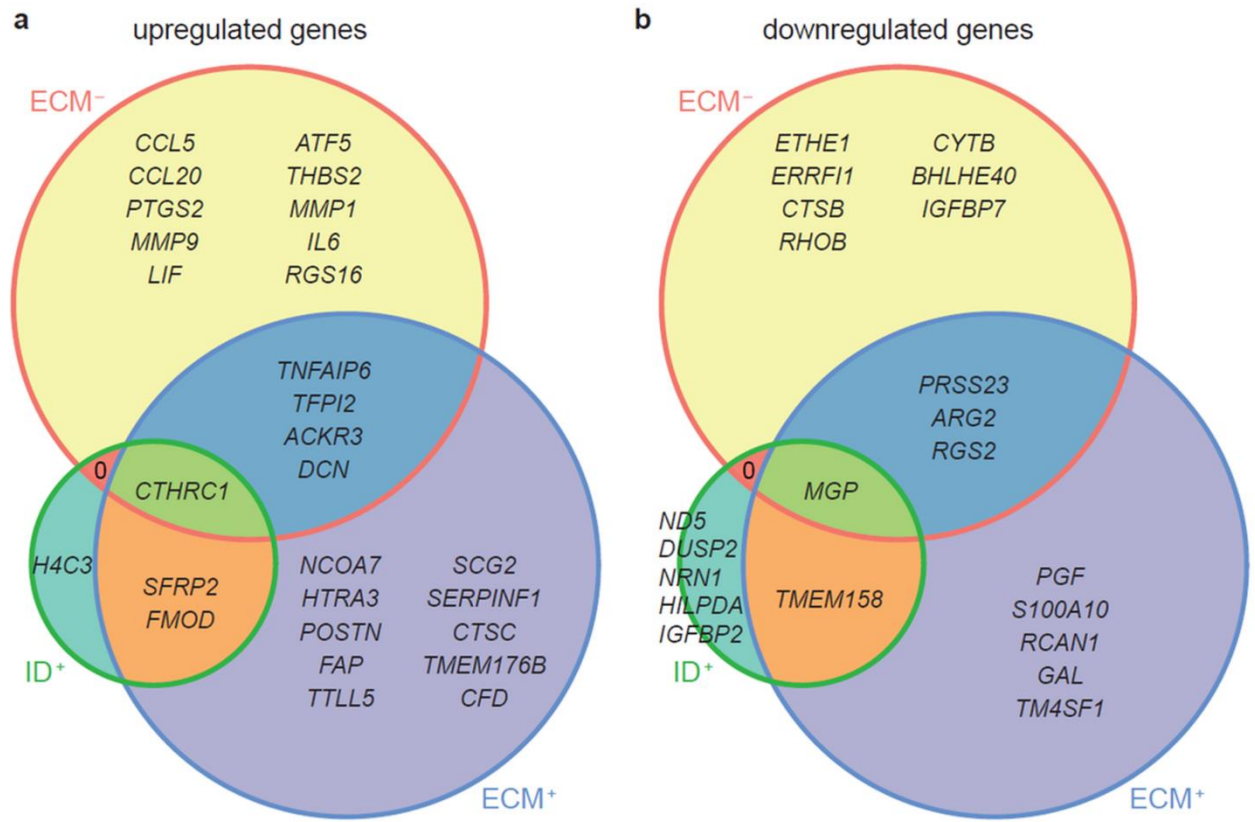
MINISTERSTVO ŠKOLSTVÍ MLÁDEŽE A TĚLOVÝCHOVY



How to distinguish them on whole genome scale? The good approach could be the Single Cell Sequencing



Zheng et al. Nature Communications 2017


a
upregulated genes
ECM⁻
CCL5
CCL20
PTGS2
MMP9
LIF
ATF5
THBS2
MMP1
IL6
RGS16
TNFAIP6
TFPI2
ACKR3
DCN
0
CTHRC1
H4C3
SFRP2
FMOD
NCOA7
HTRA3
POSTN
FAP
TTLL5
SCG2
SERPINF1
CTSC
TMEM176B
CFD
ID⁺
ECM⁺
b
downregulated genes
ECM⁻
ETHE1
ERRFI1
CTSB
RHOB
CYTB
BHLHE40
IGFBP7
PRSS23
ARG2
RGS2
0
MGP
ND5
DUSP2
NRN1
HILPDA
IGFBP2
TMEM158
PGF
S100A10
RCAN1
GAL
TM4SF1
ID⁺
ECM⁺