

Single cell sequencing

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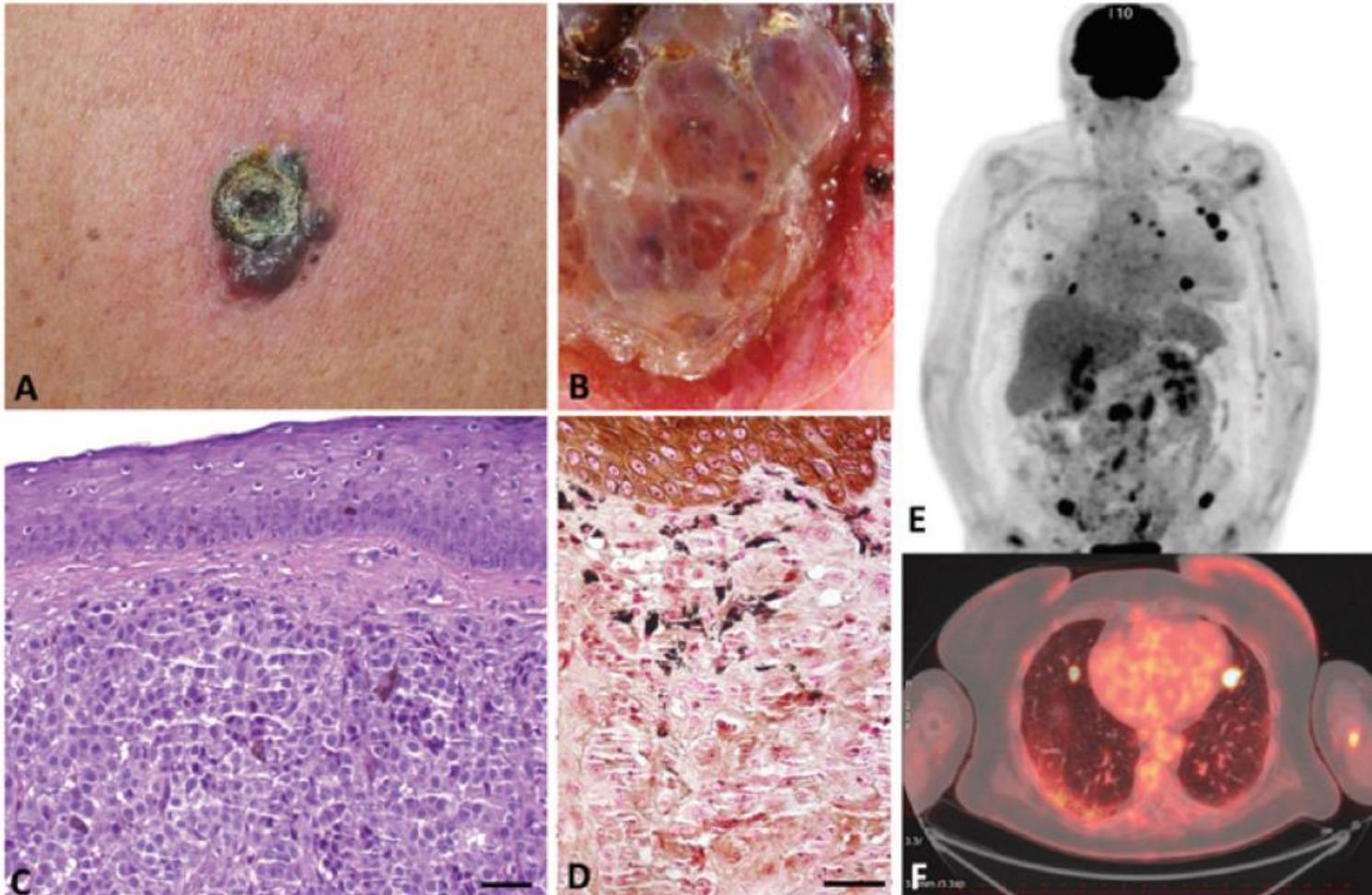
Prague and Vestec

Czech Republic

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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 ŠTORK², LUKÁŠ LACINA^{1,3} and KAREL SMETANA Jr.^{1,3}



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.

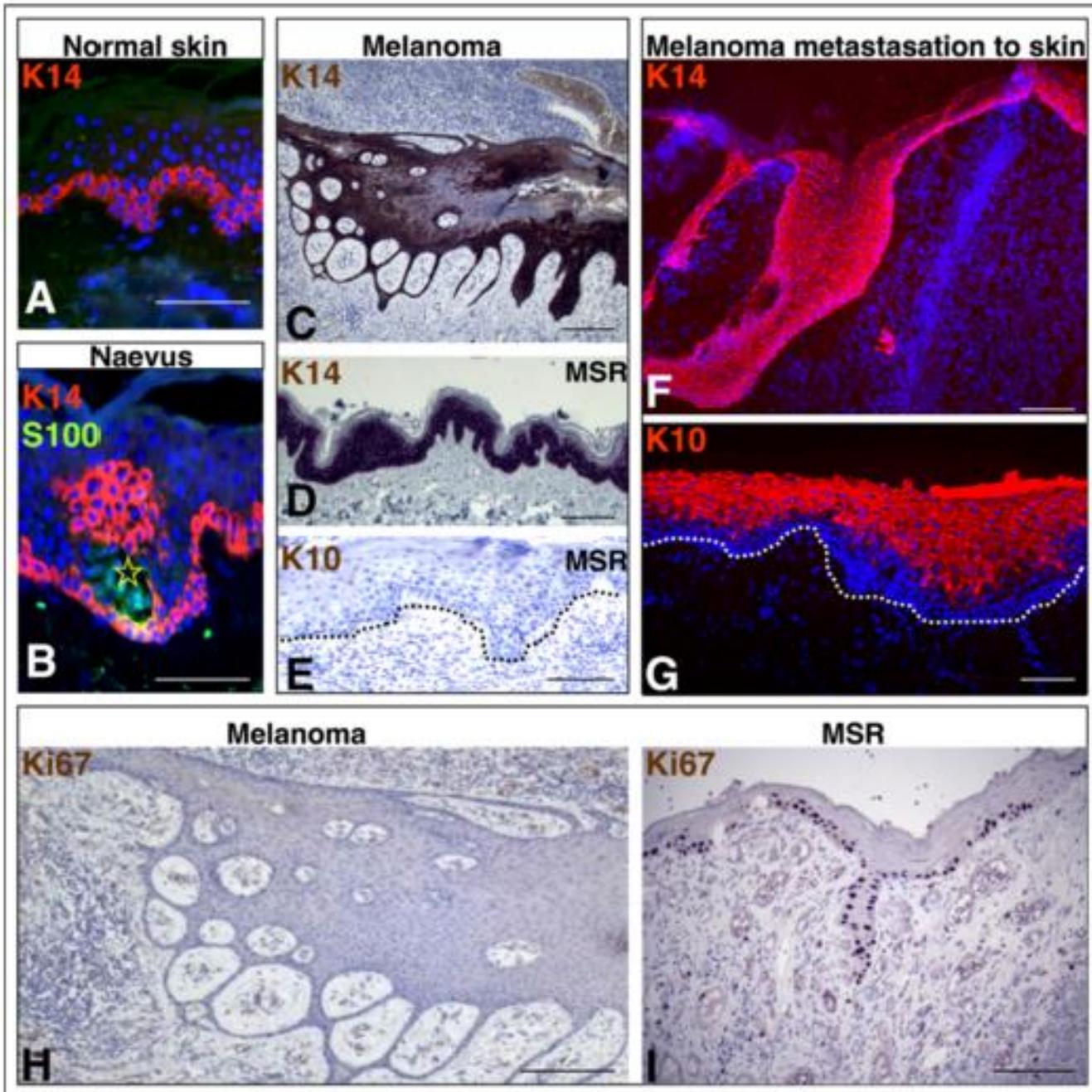
RESEARCH

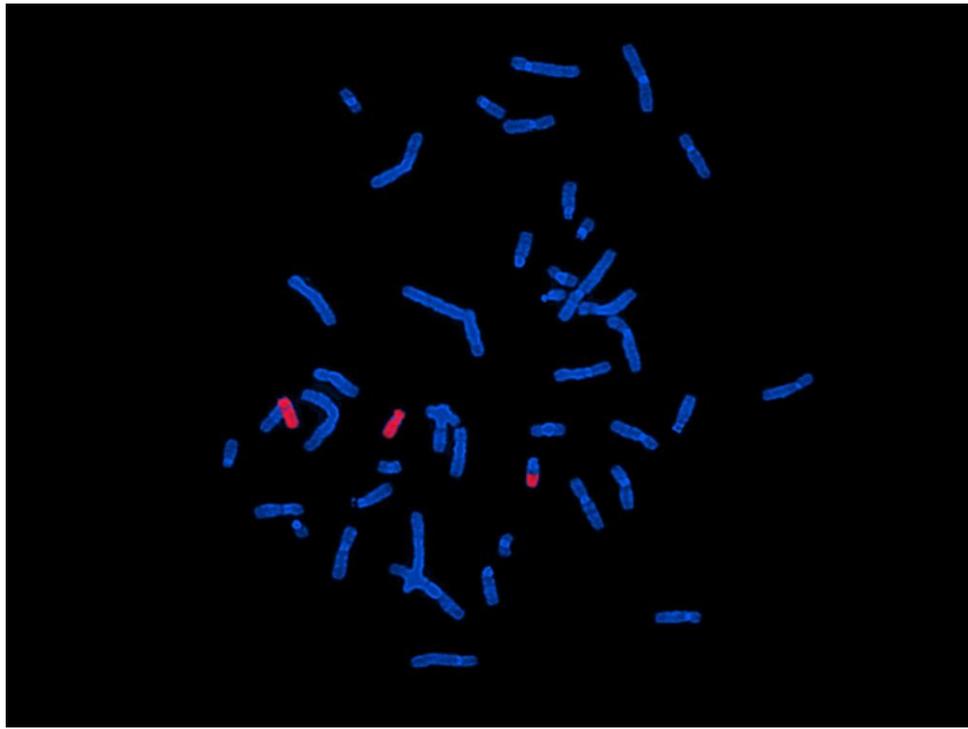
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Melanoma cells influence the differentiation pattern of human epidermal keratinocytes

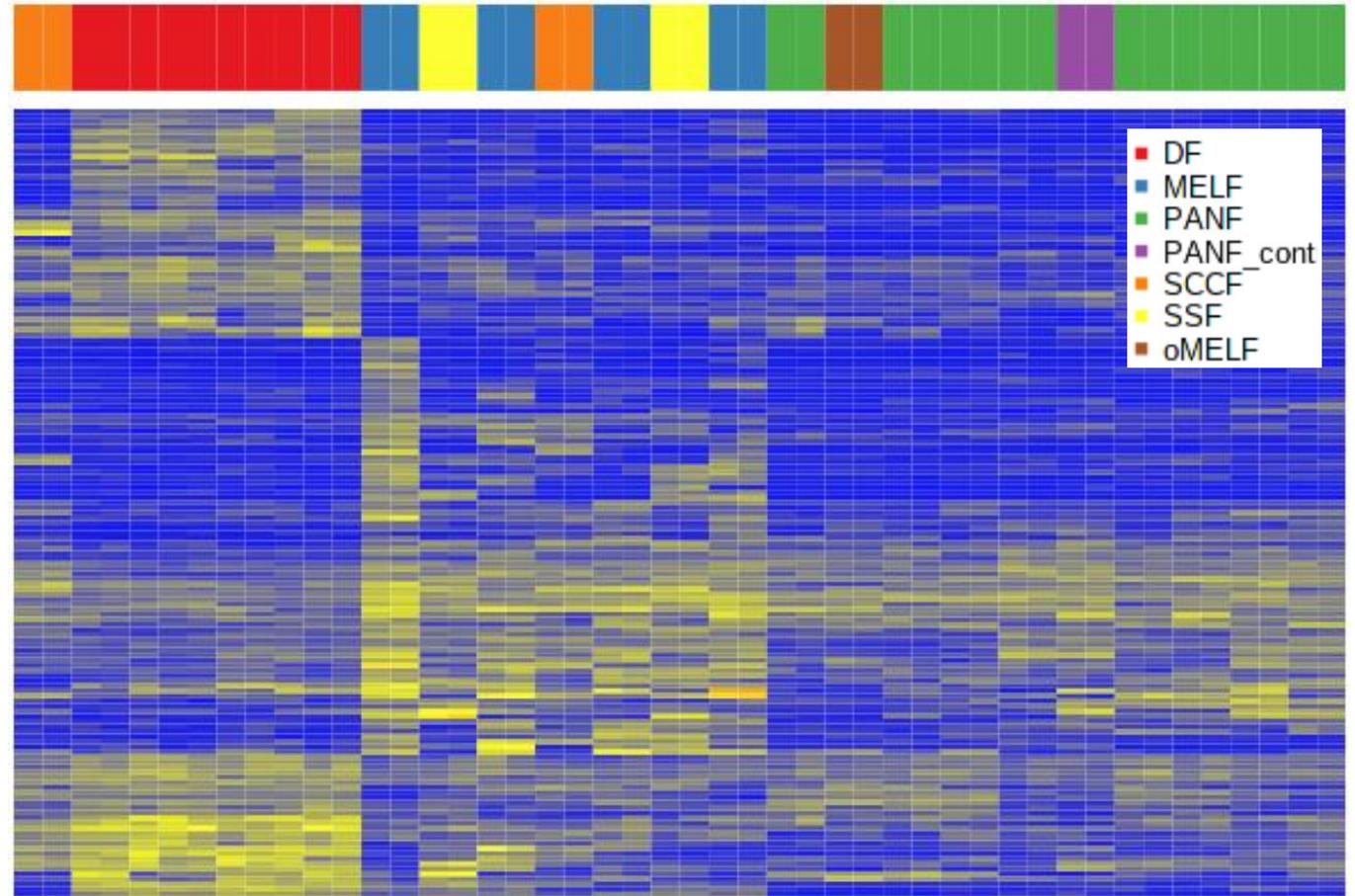
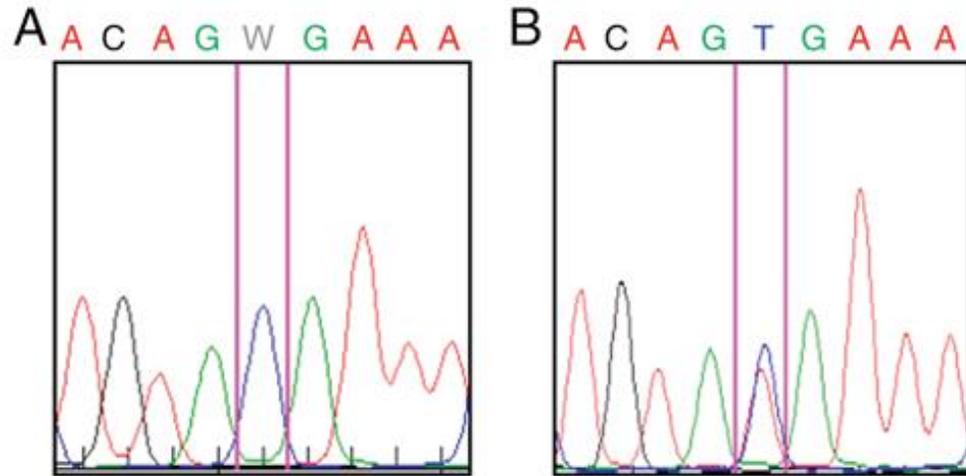
Ondřej Kodet^{1,2}, Lukáš Lacina^{1,2,3}, Eliška Krejčí¹, Barbora Dvořánková¹, Miloš Grim¹, Jiří Štokr², Daniela Kodetová⁴, Čestmír Vlček⁵, Jana Šáchová⁵, Michal Kolář⁵, Hynek Strnad^{5*} and Karel Smetana^{1*}

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





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

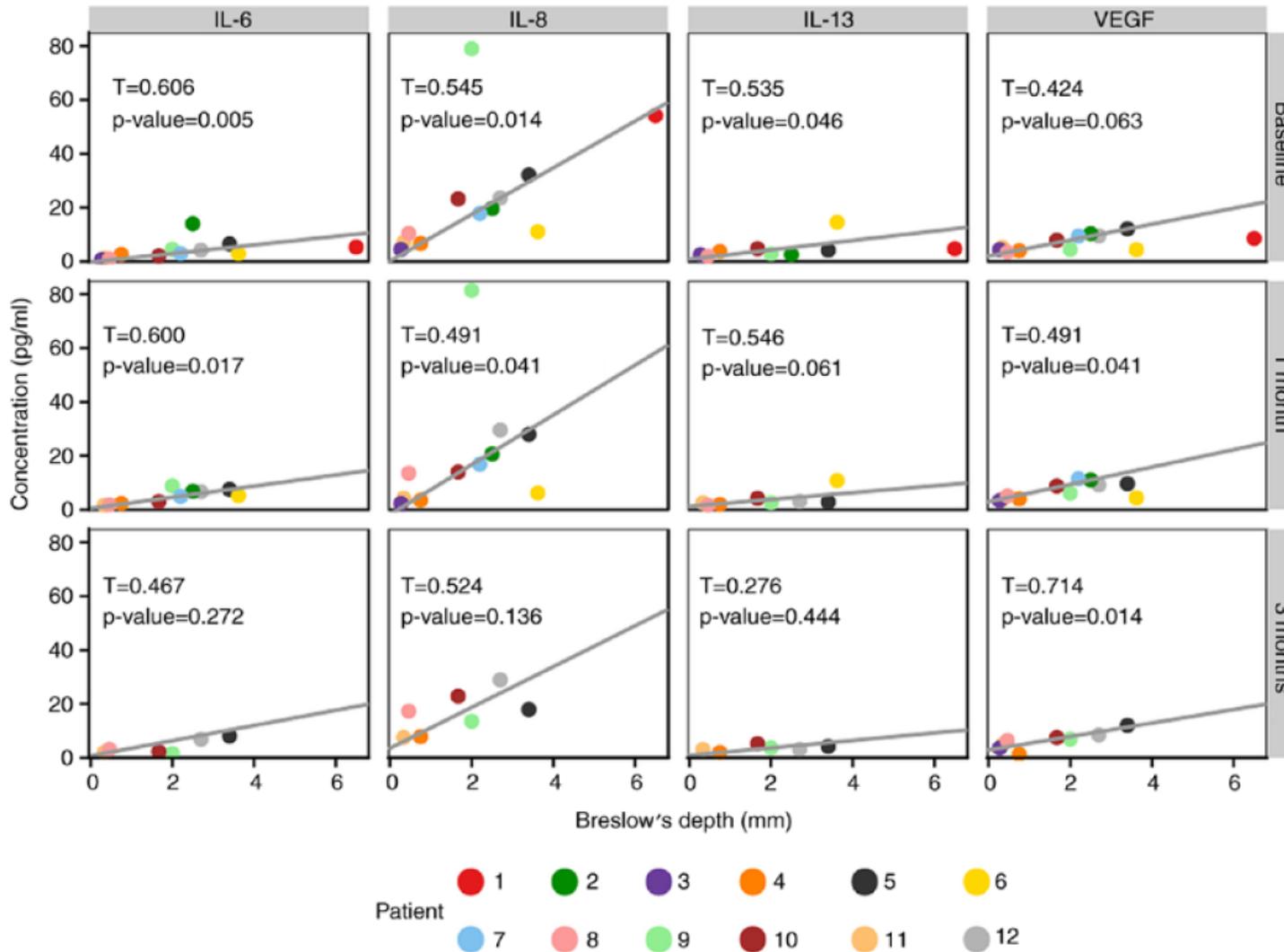


Krev	Pigmenty	Minerály	Toxikologie	Krevní obraz	Výpočty odpadů	Punktát,sonda,	Speciální koagulace	Speciální koagulace
Enzymy	<input type="checkbox"/> Bilirubin celk.	<input type="checkbox"/> Na, K, Cl	<input type="checkbox"/> Alkohol (etanol)	<input type="checkbox"/> Kapilární krev	<input type="checkbox"/> Diuréza (ml), čas	<input type="checkbox"/> drén,dialyzát	Panely	Jednotlivé metody
<input type="checkbox"/> AST	<input type="checkbox"/> Bilirubin konjug.	<input type="checkbox"/> Vápník	<input type="checkbox"/> Paracetamol	<input type="checkbox"/> Krevní obraz	Moč	<input type="checkbox"/> Rozl.trans./exsu	<input type="checkbox"/> Agreg. dest. opticky	<input type="checkbox"/> Pt-krvácivost
<input type="checkbox"/> ALT	Dusíkaté látky	<input type="checkbox"/> Fosfor anorg.	<input type="checkbox"/> Salicyláty	<input type="checkbox"/> KO+dif.rozpočet	<input type="checkbox"/> Proteinurie kvant.	<input type="checkbox"/> AMS	<input type="checkbox"/> Agreg. dest. imped.	<input type="checkbox"/> Pt-Rumpel Leede
<input type="checkbox"/> GGT	<input type="checkbox"/> Močovina (urea)	<input type="checkbox"/> Hořčík	Sérologie	<input type="checkbox"/> Dif dodatečně	<input type="checkbox"/> Protein/kreatinin	<input type="checkbox"/> LD	<input type="checkbox"/> PFA	<input type="checkbox"/> F VIII
<input type="checkbox"/> ALP	<input type="checkbox"/> Kreatinin	<input type="checkbox"/> Železo	<input type="checkbox"/> Screening hepatid	<input type="checkbox"/> Dif mikroskopicky	<input type="checkbox"/> Typ proteinurie(ELFO)	<input type="checkbox"/> Glukóza	<input type="checkbox"/> Diř. dg. patol. APTT	<input type="checkbox"/> screen. spec. inhibitor
<input type="checkbox"/> AMS	<input type="checkbox"/> Kys. močová	<input type="checkbox"/> Měď	<input type="checkbox"/> Anti-HAV celk.	<input type="checkbox"/> Retikulyocyty	<input type="checkbox"/> Albuminurie	<input type="checkbox"/> Laktát	<input type="checkbox"/> Diř. dg. patol. PT	<input type="checkbox"/> Inhibitor F VIII [BU]
<input type="checkbox"/> LPS	Bílkoviny	<input type="checkbox"/> Zinek	<input type="checkbox"/> Anti-HAV IgM	<input type="checkbox"/> Schistocyty	<input type="checkbox"/> Albumin/kreat. (ACR)	<input type="checkbox"/> Triacylglyceroly	<input type="checkbox"/> Panel průkazu LA	<input type="checkbox"/> Inhibitor F IX [BU]
<input type="checkbox"/> CHS	<input type="checkbox"/> Celk.bílkovina	<input type="checkbox"/> Selen	<input type="checkbox"/> HBsAg	<input type="checkbox"/> Osm. rezistence	<input type="checkbox"/> Bence-Jonesova bílk.	<input type="checkbox"/> Cholesterol	<input type="checkbox"/> Trombotický panel	<input type="checkbox"/> vWF aktivita
<input type="checkbox"/> CK	<input type="checkbox"/> Albumin	<input type="checkbox"/> Osmolalita	<input type="checkbox"/> Anti-HBs	<input type="checkbox"/> PINK test	AMS	<input type="checkbox"/> Celk.bílkovina	<input type="checkbox"/> Panel vWillebrand. ch	<input type="checkbox"/> vWF antigen
<input type="checkbox"/> LD	<input type="checkbox"/> ELFO bílkovin	Léky	<input type="checkbox"/> Anti-HBc celk.	<input type="checkbox"/> Trombo v TRK	<input type="checkbox"/> Močovina (urea)	<input type="checkbox"/> Albumin		<input type="checkbox"/> F II
Sacharidový metab.	<input type="checkbox"/> M - protein	<input type="checkbox"/> Digoxin	<input type="checkbox"/> Anti-HBc IgM	<input type="checkbox"/> Trombo TromboExact	<input type="checkbox"/> Kreatinin	<input type="checkbox"/> Močovina (urea)	Doplňující vyšetření	<input type="checkbox"/> F V
<input type="checkbox"/> Glukóza	<input type="checkbox"/> Volné lehké řetězce	<input type="checkbox"/> Teofylin	<input type="checkbox"/> HBeAg	Koagulace	<input type="checkbox"/> Clearance kreat	<input type="checkbox"/> Kys. močová	<input type="checkbox"/> Souhlasím s úpravou	<input type="checkbox"/> F VII
<input type="checkbox"/> Glukóza v plazmě	<input type="checkbox"/> Prealbumin	<input type="checkbox"/> Gentamicin před	<input type="checkbox"/> Anti-HBe	<input type="checkbox"/> Koagulační scr.	<input type="checkbox"/> Na, K, Cl	<input type="checkbox"/> Vápník	<input type="checkbox"/> požadovaných metod	<input type="checkbox"/> F X
<input type="checkbox"/> K.mléčná (laktát)	<input type="checkbox"/> Transferin	<input type="checkbox"/> Gentamicin po	<input type="checkbox"/> Anti-HCV	<input type="checkbox"/> APTT	<input type="checkbox"/> Vápník	<input type="checkbox"/> Fosfor anorg.	Druh protidest. léčby	<input type="checkbox"/> F XI
<input type="checkbox"/> Glyk.Hb (HbA1c)	<input type="checkbox"/> Feritin	<input type="checkbox"/> Amikacin před	Hormony, vitaminy, AK	<input type="checkbox"/> Protrombin. čas	<input type="checkbox"/> pH	<input type="checkbox"/> Hořčík	<input type="checkbox"/> ASA	<input type="checkbox"/> F XII
Lipidový metab.	<input type="checkbox"/> Solubilní TfR	<input type="checkbox"/> Amikacin po	<input type="checkbox"/> hCG celkový	<input type="checkbox"/> Trombinový čas	<input type="checkbox"/> KO v jiných mat.	<input type="checkbox"/> Měď	<input type="checkbox"/> Clopidogrel	<input type="checkbox"/> F XIII
<input type="checkbox"/> Screening lipidů	<input type="checkbox"/> CDT (bezсах. tř.)	<input type="checkbox"/> Vankomycin před	<input type="checkbox"/> sFlt-1	<input type="checkbox"/> Fibrinogen	<input type="checkbox"/> KO+dif. v jiných	<input type="checkbox"/> Zinek	<input type="checkbox"/> Ostatní	<input type="checkbox"/> Kaolinový test
<input type="checkbox"/> Triacylglyceroly	<input type="checkbox"/> Prokalcitonin	<input type="checkbox"/> Vankomycin po	<input type="checkbox"/> PIGF	<input type="checkbox"/> Antitrombin	Mozkomišní mok	<input type="checkbox"/> Selen	<input type="checkbox"/> Žádná	<input type="checkbox"/> Reptilázový test
<input type="checkbox"/> Cholesterol celk.	<input type="checkbox"/> CRP	<input type="checkbox"/> Fenobarbital	<input type="checkbox"/> NT-proBNP	<input type="checkbox"/> D-dimery	<input type="checkbox"/> Likvor - základn	<input type="checkbox"/> Osmolalita	<input type="checkbox"/> Nelze zjistit	<input type="checkbox"/> dRVVT
<input type="checkbox"/> HDL cholesterol	<input type="checkbox"/> Myoglobin	<input type="checkbox"/> Fenytoin	<input type="checkbox"/> Erytropoetin	<input type="checkbox"/> Reptilázový test	<input type="checkbox"/> Likvor - výpočty	<input type="checkbox"/> Moč chem. a sed.		<input type="checkbox"/> ProC global
<input type="checkbox"/> LDL cholesterol	<input type="checkbox"/> Troponin T	<input type="checkbox"/> Karbamazepin	<input type="checkbox"/> Vitamin B12	<input type="checkbox"/> Korekce APTT	Spektrofotometri	Toxikologie	Druh antikoag. léčby	<input type="checkbox"/> Protein C aktivita
<input type="checkbox"/> Apo A-I	<input type="checkbox"/> alfa1-antitrypsin	<input type="checkbox"/> Valproát	<input type="checkbox"/> Kys.listová (folát)	<input type="checkbox"/> Korekce PT	<input type="checkbox"/> Oligoklonální IgG	<input type="checkbox"/> Screening T10	<input type="checkbox"/> Warfarin	<input type="checkbox"/> Protein S aktivita
<input type="checkbox"/> Apo B	<input type="checkbox"/> Haptoglobin	<input type="checkbox"/> Levetiracetam	<input type="checkbox"/> Homocystein celk.	<input type="checkbox"/> APTT aktin	<input type="checkbox"/> Průkaz likvorey	<input type="checkbox"/> Screening T6	<input type="checkbox"/> Heparin	<input type="checkbox"/> Protein C antigen
<input type="checkbox"/> Lp(a)	<input type="checkbox"/> Ceruloplazmin	<input type="checkbox"/> Lamotrigin		<input type="checkbox"/> Euglob.fibrinolýza	<input type="checkbox"/> Beta-trace protein	<input type="checkbox"/> Fentanyl	<input type="checkbox"/> LMWH	<input type="checkbox"/> Protein S antigen
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	<input type="checkbox"/> Cystatin C	<input type="checkbox"/> Takrolimus		<input type="checkbox"/> LMWH (antiXa)			<input type="checkbox"/> Žádná	<input type="checkbox"/> Agreg. po kolagenu
		<input type="checkbox"/> Sirolimus		<input type="checkbox"/> Pradaxa (dTT)			<input type="checkbox"/> Nelze zjistit	<input type="checkbox"/> Agreg. po kys. arachid.
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		<input type="checkbox"/> Kofein						<input type="checkbox"/> ASPI
								<input type="checkbox"/> TRAP

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

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 LACINA¹⁻³,
 IVANA KRAJSOVÁ¹, JIŘÍ ŠTORK¹, HANA KOVÁŘOVÁ⁴, HELENA KUPCOVÁ SKALNÍKOVÁ⁴,
 PETR VODIČKA⁴, JAN MOTLÍK⁴, PAVEL DUNDR⁵, KAREL SMETANA Jr^{2,3} and ONDŘEJ KODET¹⁻³

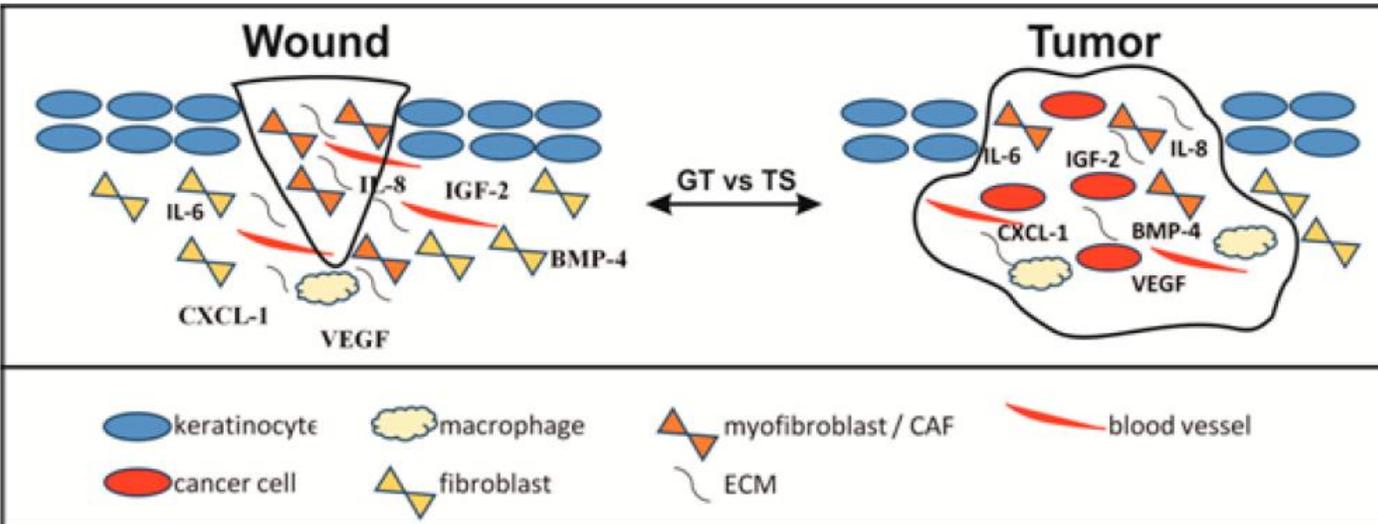


Level of inflammation-supporting markers in serum of patients with CMM reflects the Breslow's depth in mm.

Review

How Signaling Molecules Regulate Tumor Microenvironment: Parallels to Wound Repair

Peter Gál ^{1,2,3,*}, Lenka Varinská ^{1,2}, Lenka Fáber ², Štěpán Novák ^{4,5}, Pavol Szabo ^{1,4,6}, Petra Mítrenková 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.

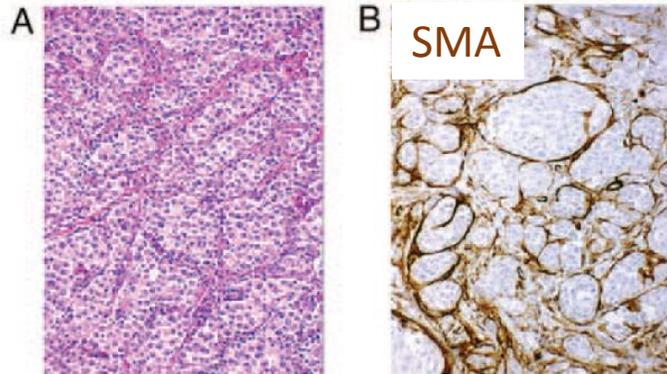
Fibroblasts potentiate melanoma cells in vitro invasiveness induced by UV-irradiated keratinocytes

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 Strnad⁶ · Michal Kolář⁶ · Aleksi Šedo⁷ · 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

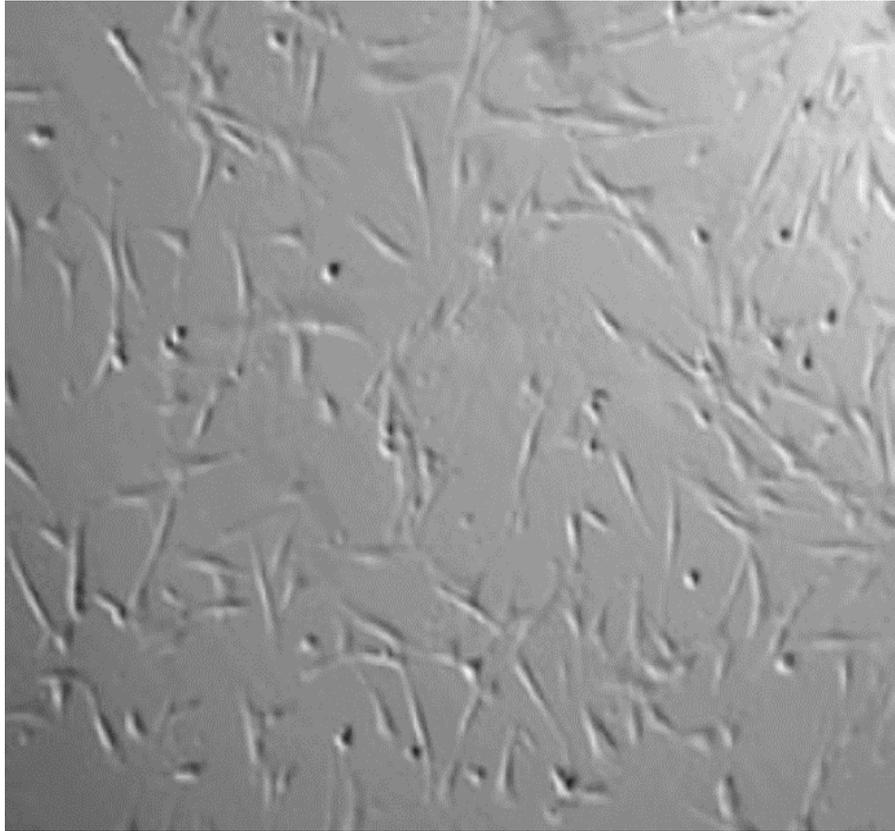
ONDŘEJ KODET^{1,4} · BARBORA DVOŘÁNKOVÁ^{1,3} · BĚLA BENDLOVÁ⁵ · VLASTA SÝKOROVÁ⁵ · IVANA KRAJSOVÁ⁴ · JIŘÍ ŠTORK^{2,4} · JAN KUČERA^{1,2,4} · PAVOL SZABO^{1,3} · HYNEK STRNAD⁶ · MICHAL KOLÁŘ⁶ · ČESTMÍR VLČEK⁶ · KAREL SMETANA Jr.^{1,3} and LUKÁŠ LACINA¹⁻⁴



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 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		

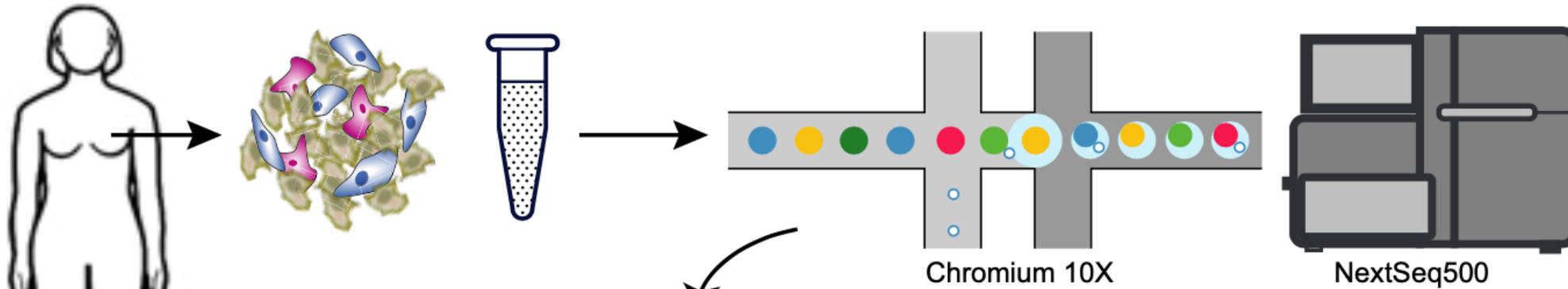
CAF produce many factors supporting inflammation.

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

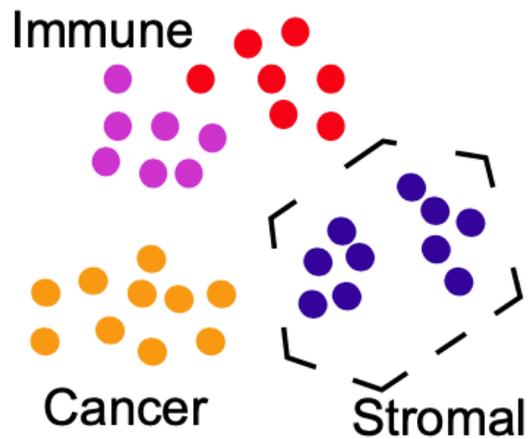


Tissue Dissociation

Unbiased Single-Cell RNA Sequencing



Analysis

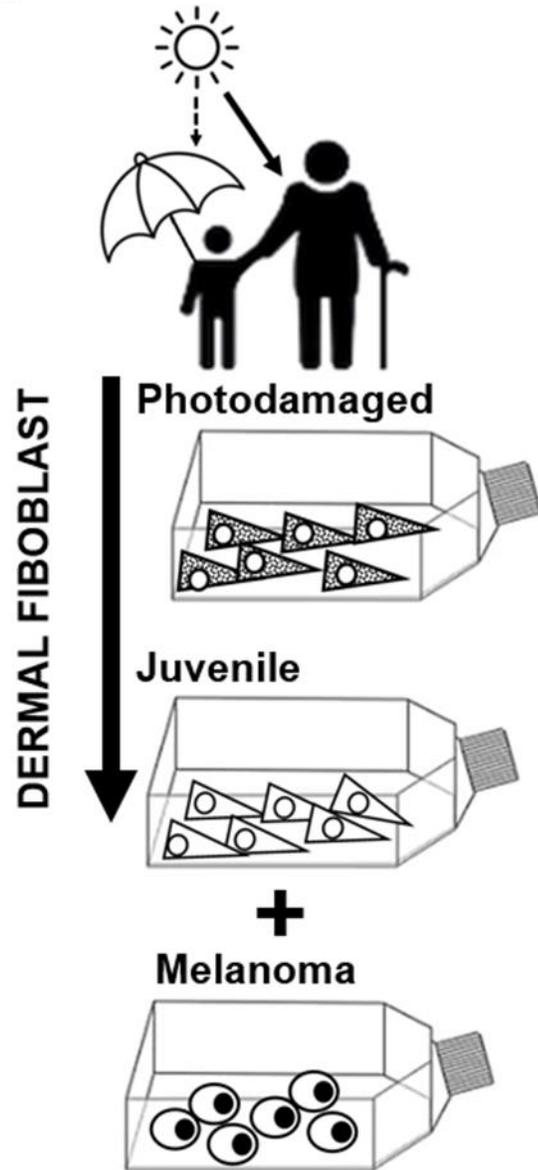


Every single cell is encapsulated in a lipid droplet and

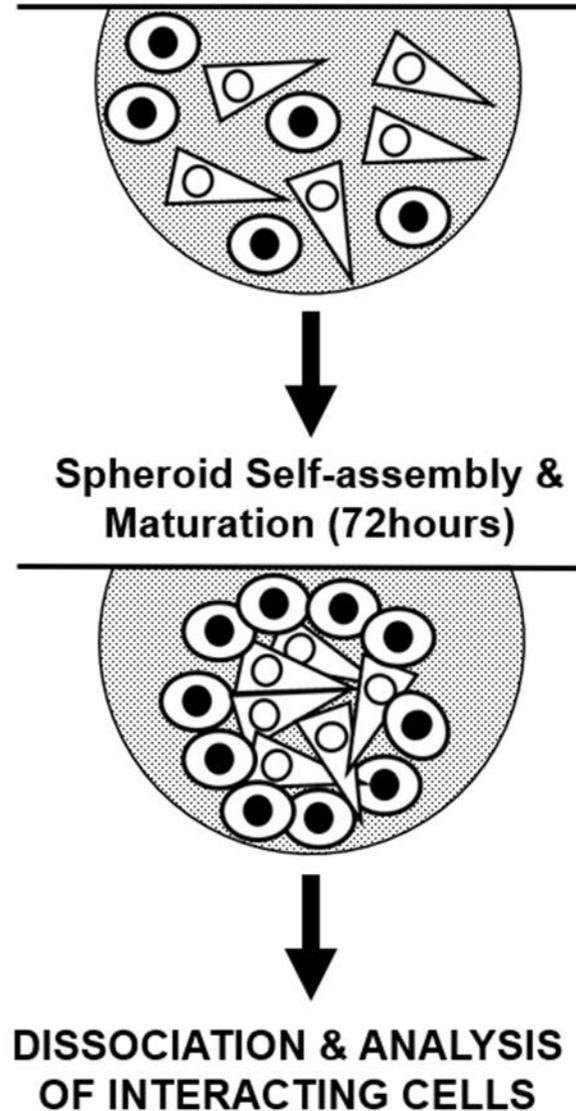
1. DNA copy number variation can be analyzed.
2. mRNA is reversely transcribed to DNA, amplified and analyzed.

Bioinformatic analysis is an indispensable part of the experiment.

A) Cell Isolation



B) Heterogeneous Spheroid Formation (hanging drop)



Article

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

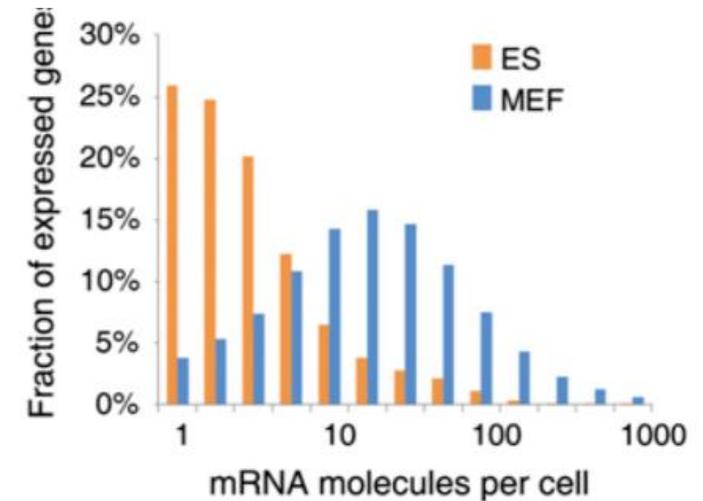
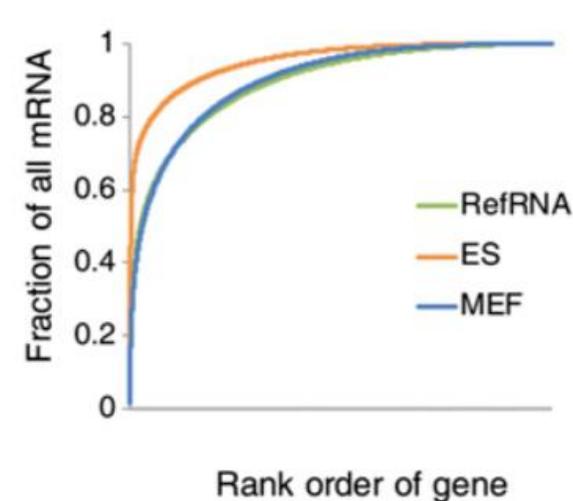
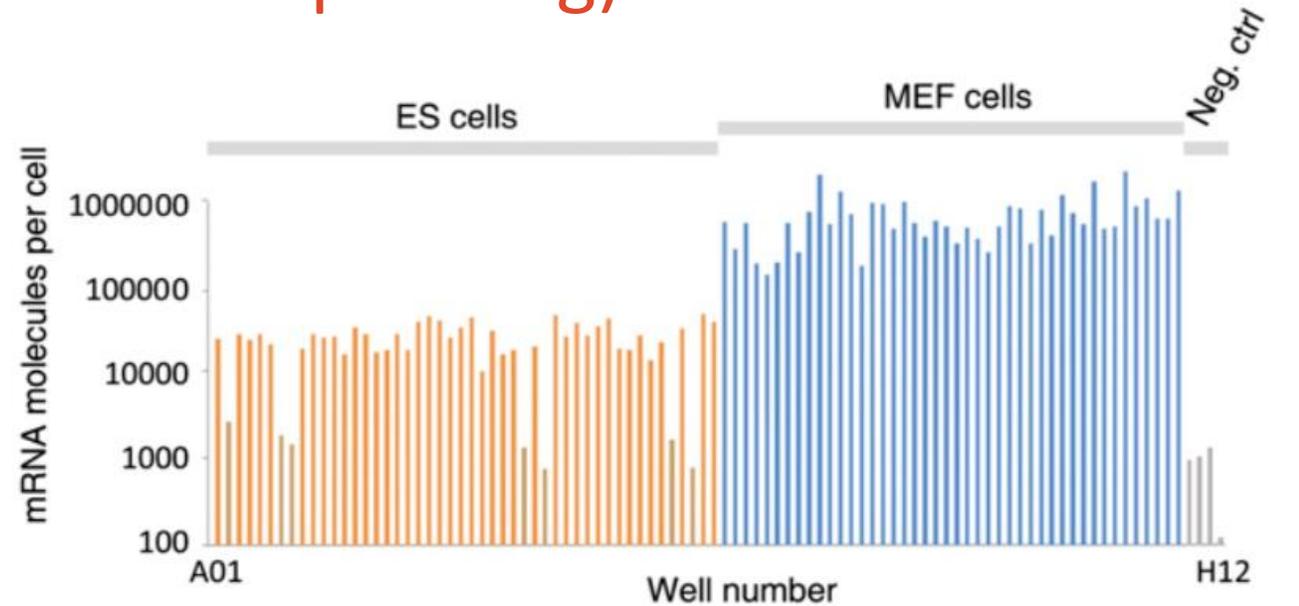
Jiří Novotný ^{1,2,†}, Karolína Strnadová ^{3,4,†}, Barbora Dvořánková ^{3,4}, Šárka Kocourková ¹, Radek Jakša ⁵, Pavel Dundr ⁵, Václav Pačes ¹, Karel Smetana Jr ^{3,4}, Michal Kolář ^{1,*} and Lukáš Lacina ^{3,4,6,*}

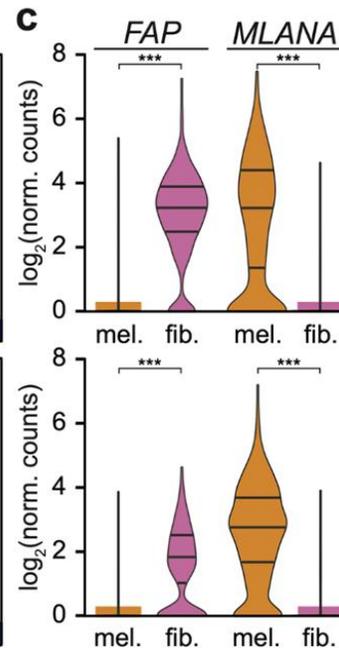
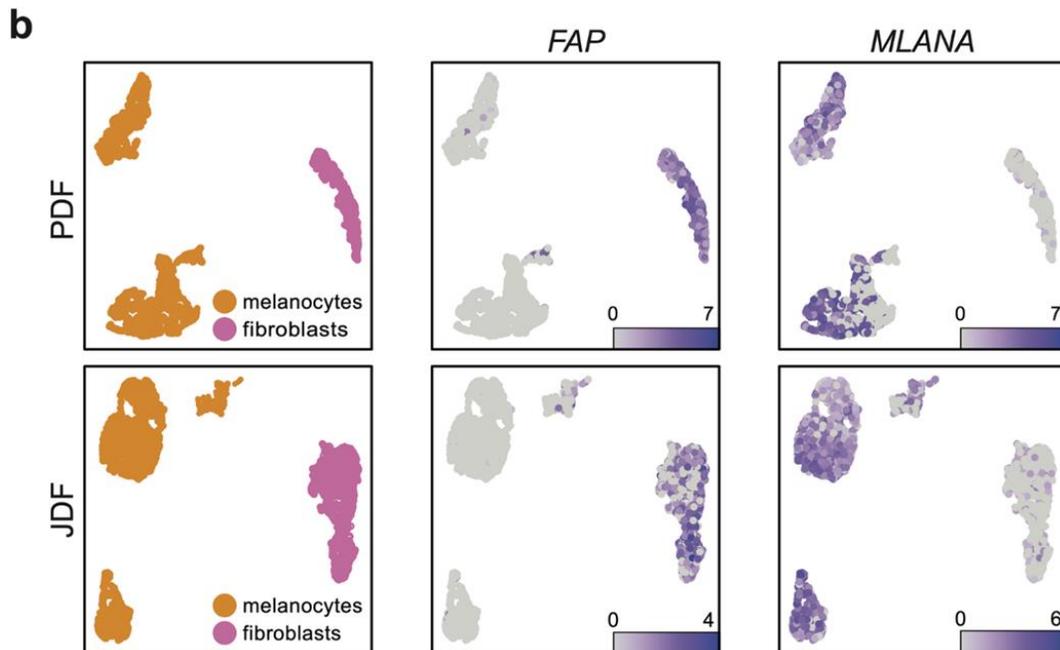
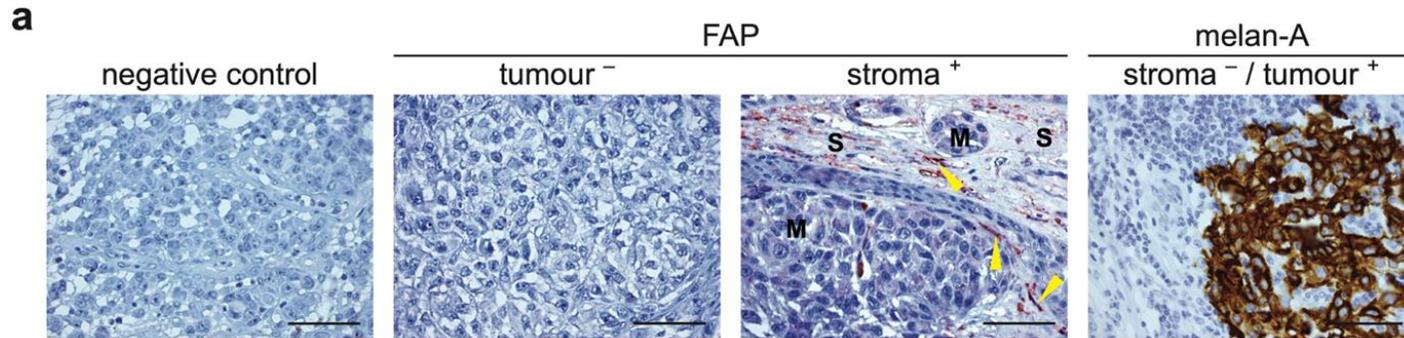
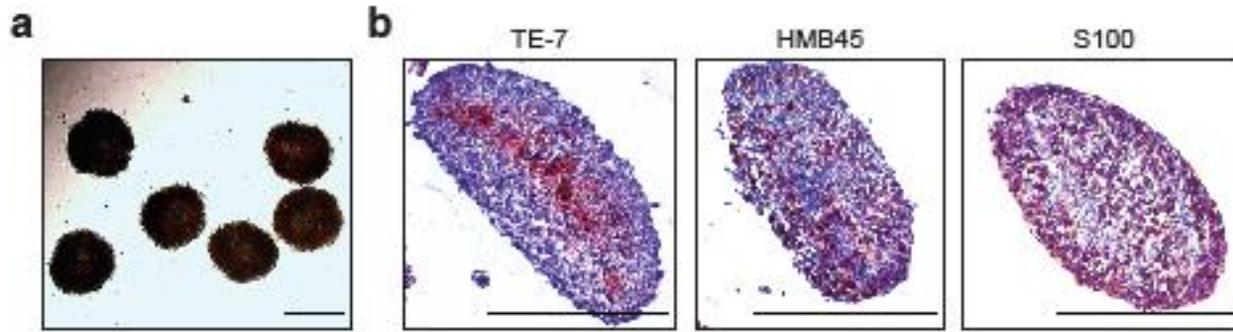
Cancers **2020**, *12*, 3324; doi:10.3390/cancers12113324

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)

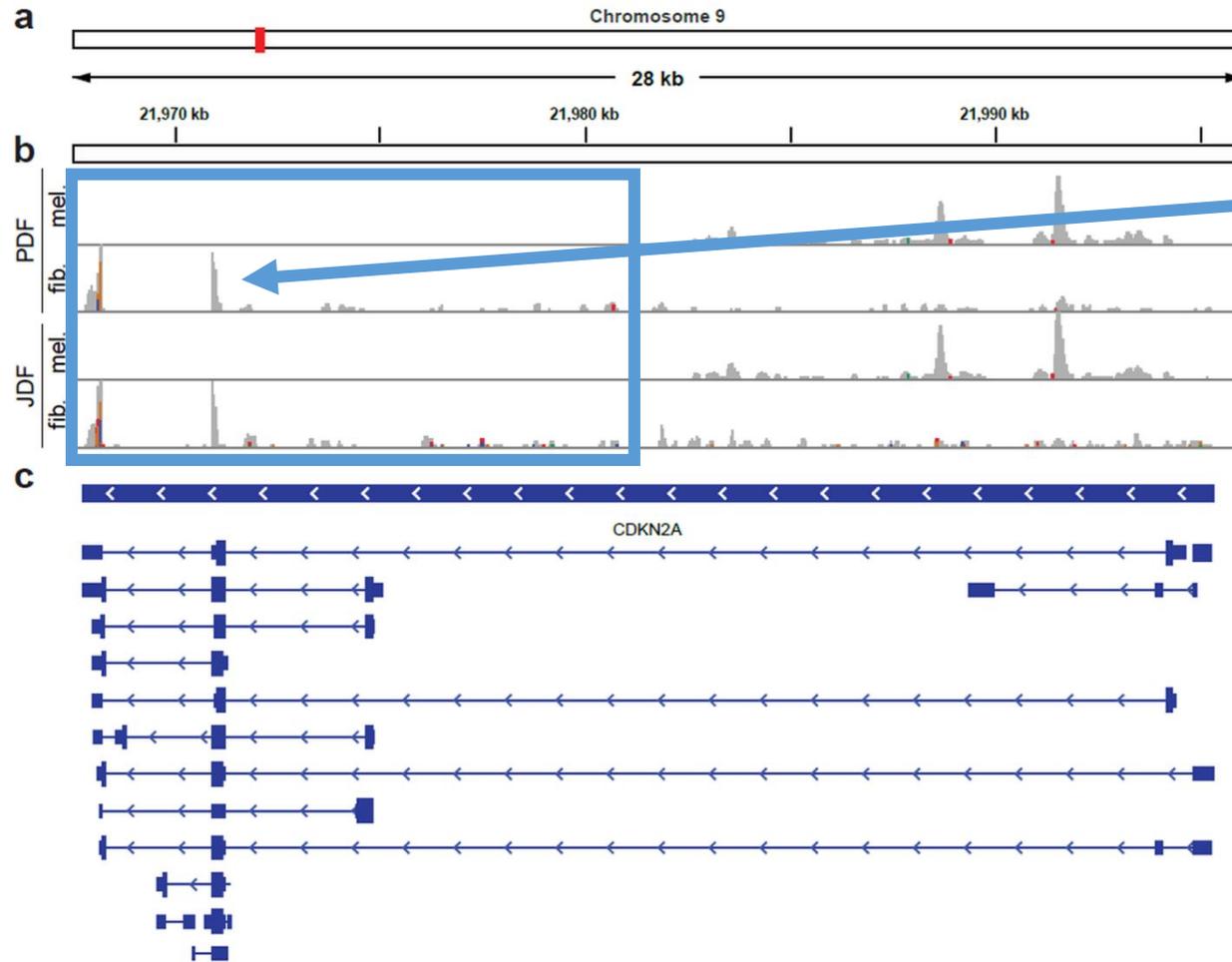
- **Library prep:** target 4 thousand cells per sample
- **Sequencing:** at least 25 thousand reads per cell
- **Cost:** expect high ten thousands CZK per sample



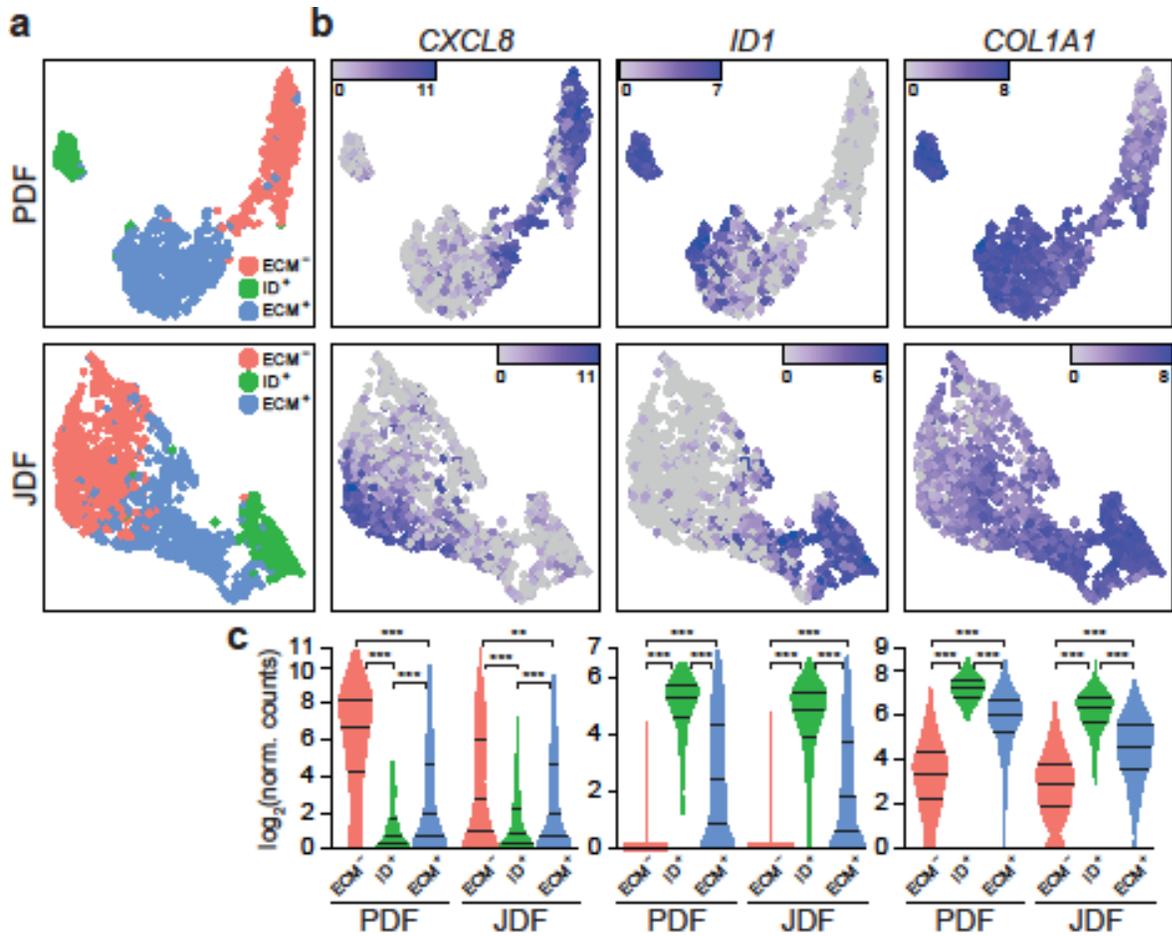


The heterogeneous spheres containing melanoma cells (G361), adult (PDF) or juvenile (JDF) fibroblasts were prepared. The cell types are diagnosed according to expression of melanoma gene (*MLANA*) and CAF gene (*FAP*) by scRNA-seq analysis.

Supplementary Figure S3

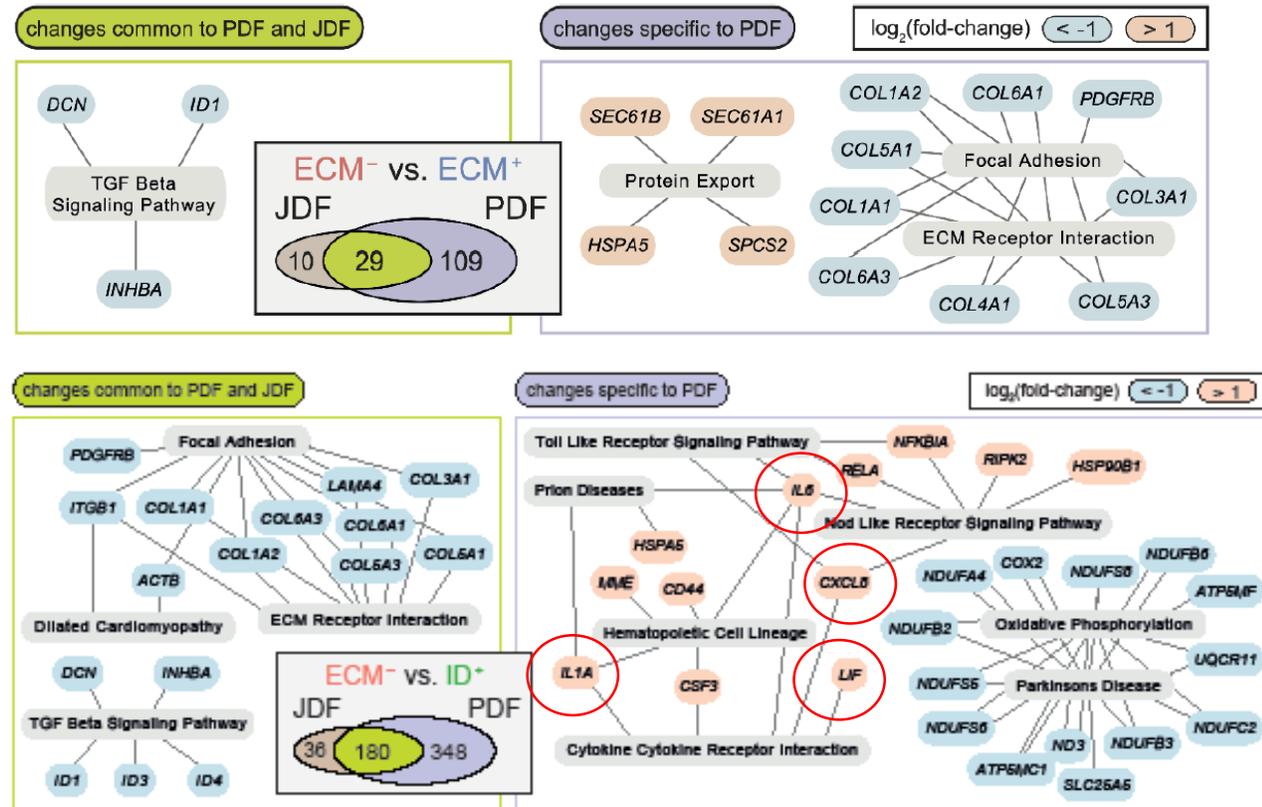


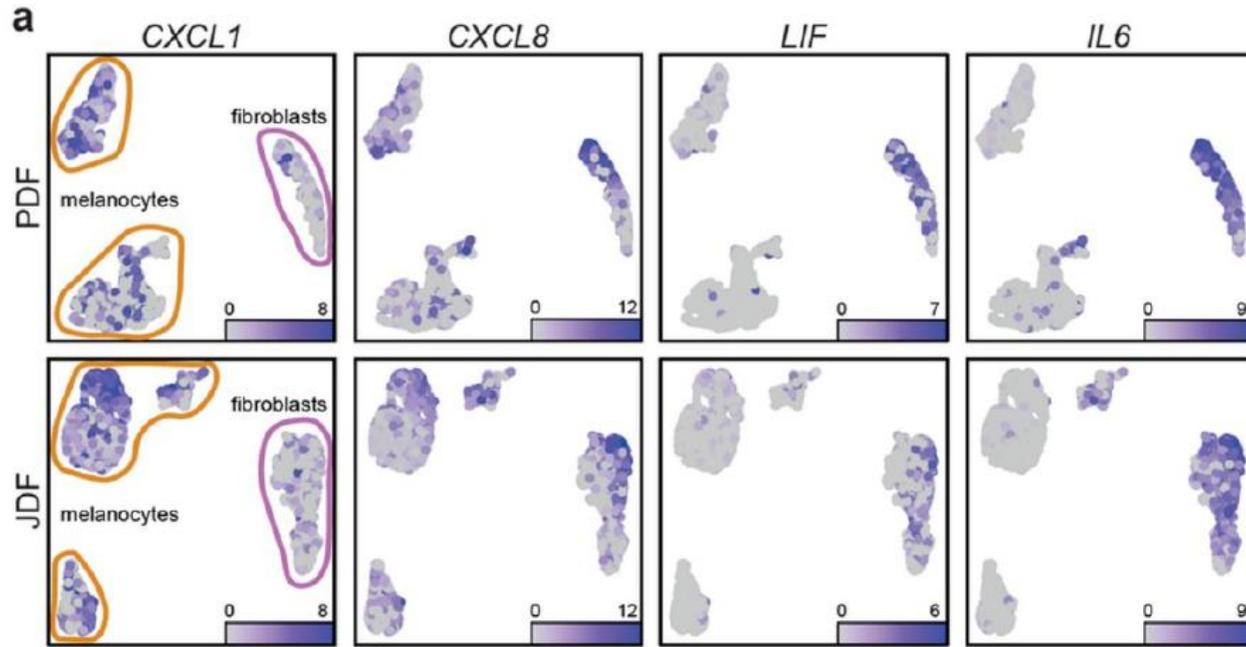
G361 CMM cells have defect of *CDKN2A* gene that is normal in both types of fibroblasts



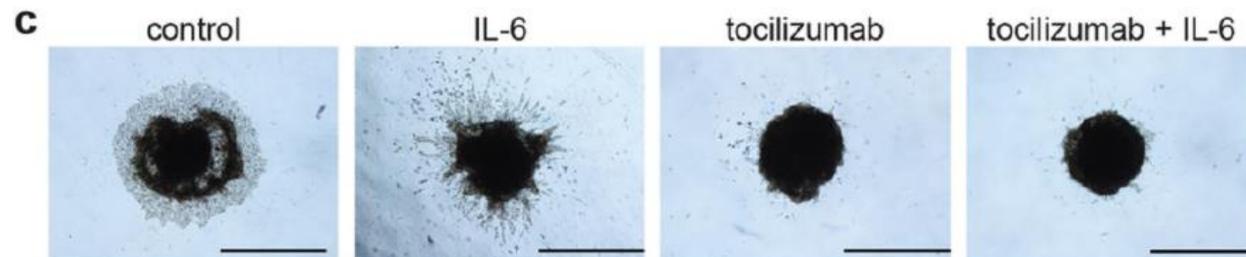
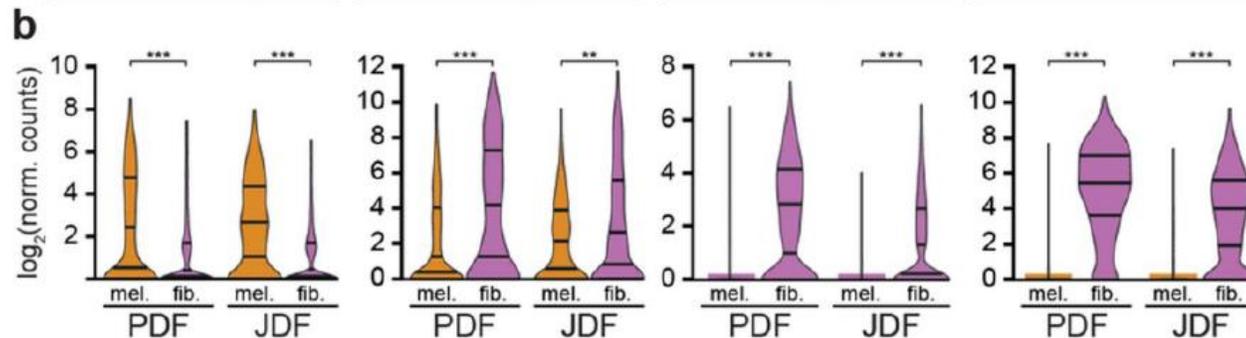
Both types of fibroblasts in spheres form heterogeneous population of cells that differ by activity of genes encoding ECM (*COL1A1*), or inflammation supporting factors (*CXCL8*) and *ID* genes depending on TGF- β signaling.

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.





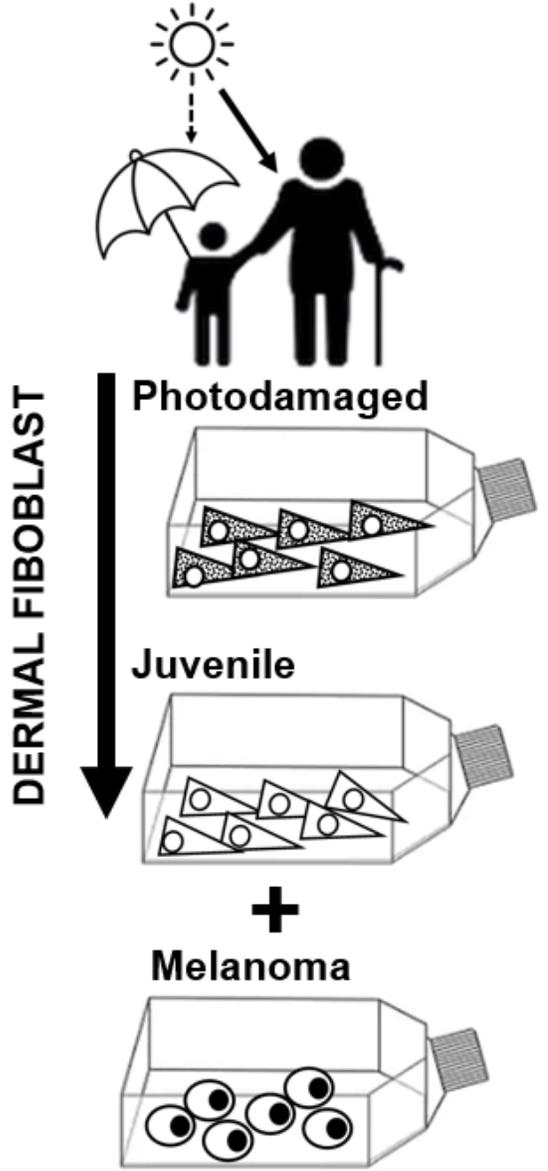
While gene for chemokine *CXCL1* is more expressed by melanoma cells, gene for chemokine *CXCL8* is expressed by both fibroblasts and melanoma cells. Genes for *LIF* and *IL6* are strongly expressed by fibroblasts.



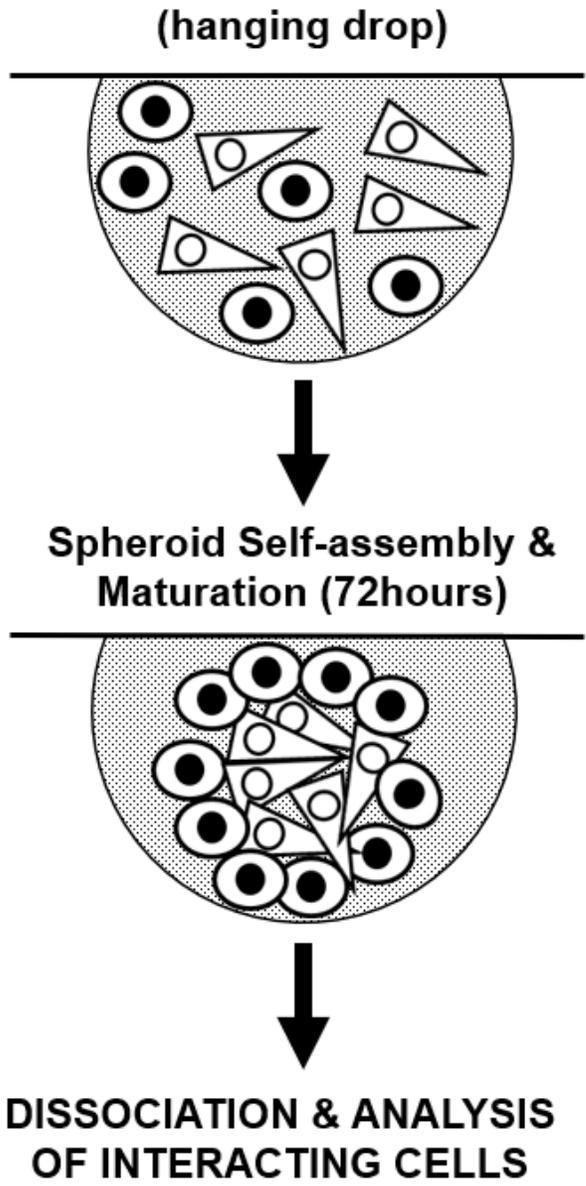
Humanized anti-IL-6R antibody
Tocilizumab inhibits migration of melanoma cells in 3D collagen gel.

Heterogeneity of the Stromal Fibroblast in Cutaneous Melanoma Spheroids

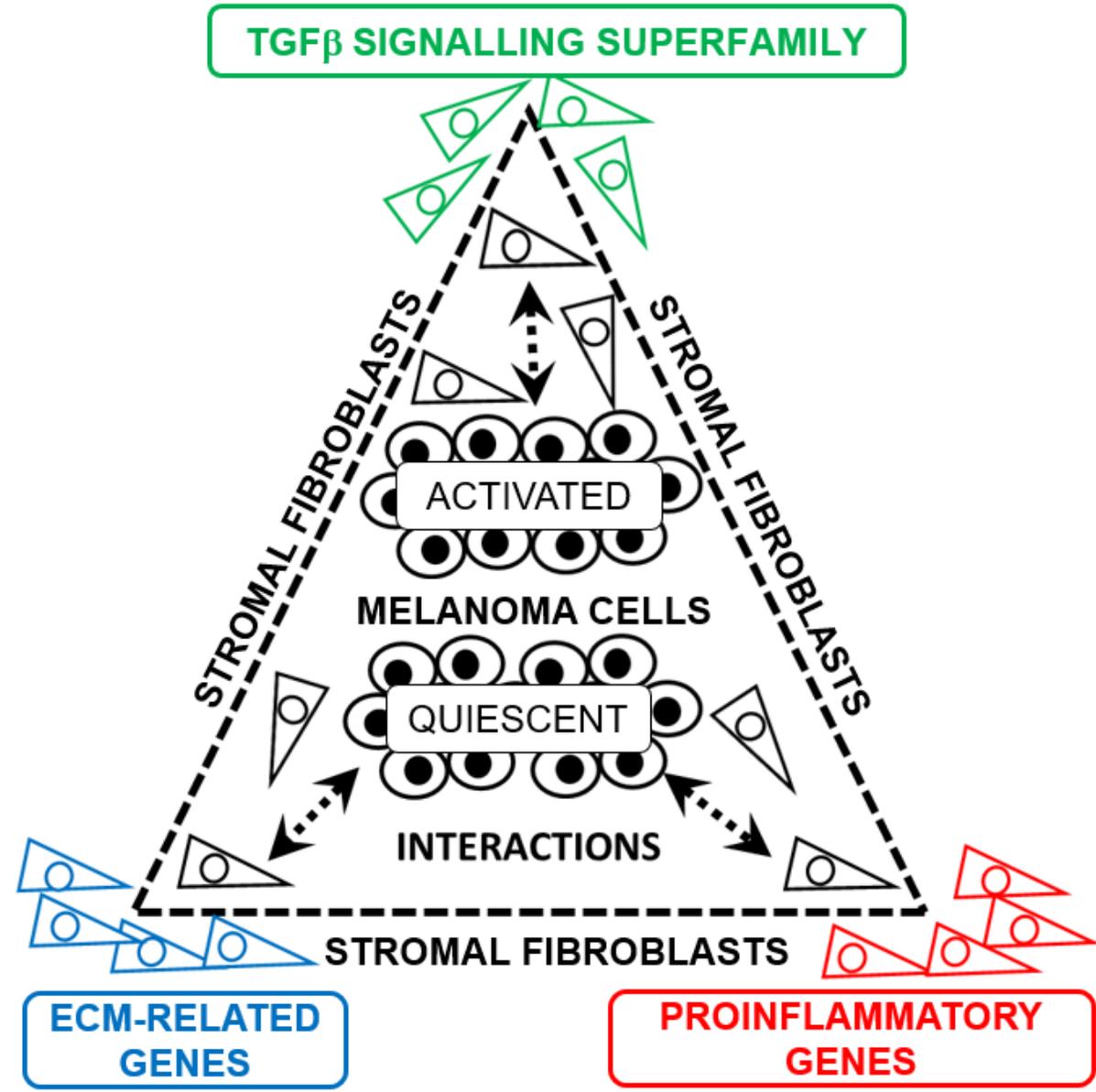
A) Cell Isolation



B) Heterogeneous Spheroid Formation



C) Single-cell RNA Sequencing & Bioinformatics



scRNA-seq represents a 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

Anatomický ústav a BIOCEV: B.Dvořánková, K.Strnadová, *H.Stýblová*



Ústav patologie: P.Dundr, R.Jakša



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



Finanční podpora:



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Centre for Tumour Ecology

Scientific coordinator of project - Prof Karel Smetana, MD, DSc.

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NEWS

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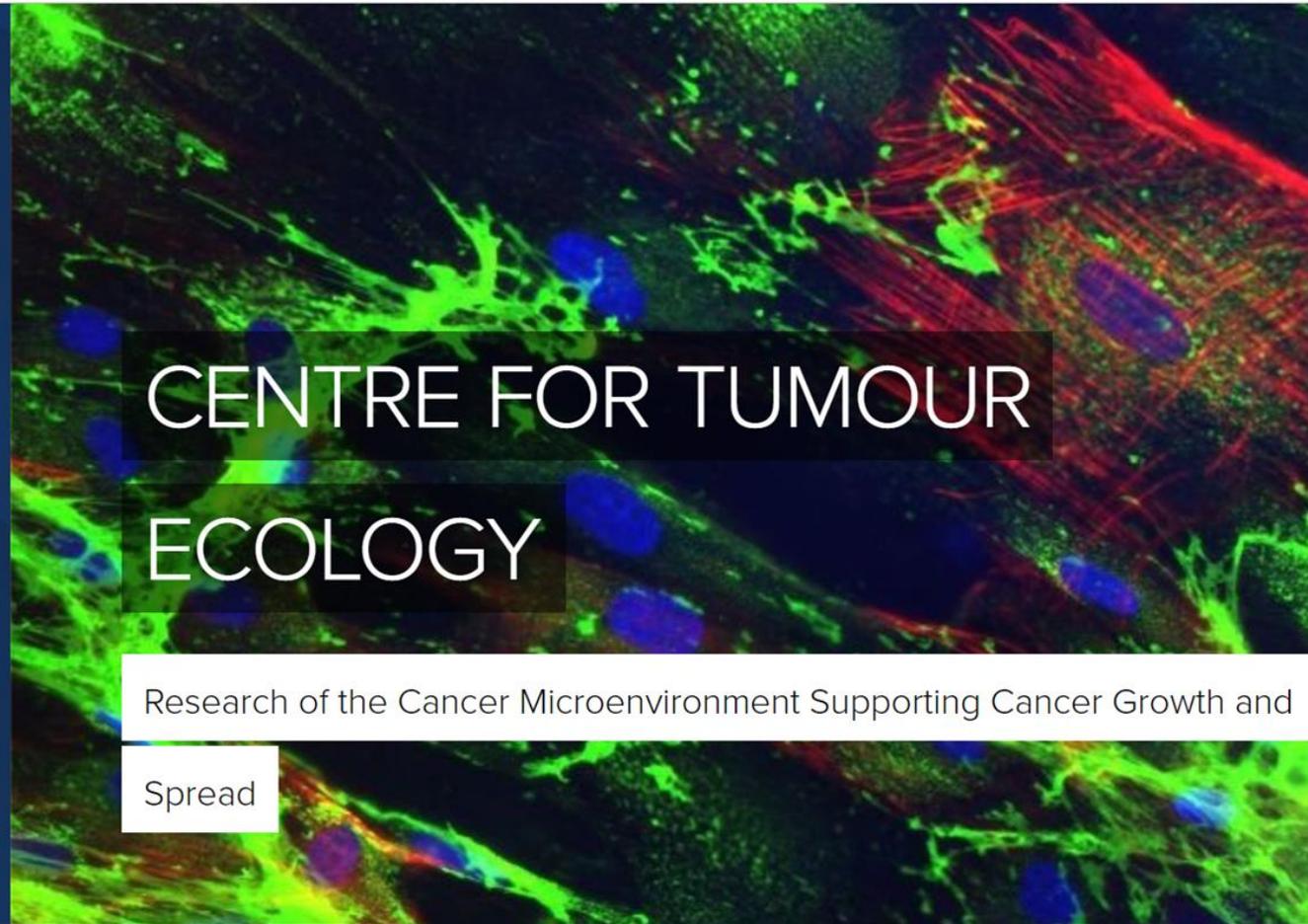
PARTICIPATING INSTITUTIONS

RESEARCH TEAMS

PROJECTS

Languages: [Čeština](#) / [English](#)

Vytvořeno službou [Webnode](#)



CENTRE FOR TUMOUR ECOLOGY

Research of the Cancer Microenvironment Supporting Cancer Growth and Spread

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

