The role of skin specific genes in skin cancer

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Molecular Biotechnology B.Sc, Ruprecht Karls University of Heidelberg, SS21

Dinkelacker, 2007. A database of genes that are expressed in a tissue-restricted manner to analyse promiscous gene expression in medullary thymic epithelial cells. Diplomarbeit, Albert-Ludwigs-Universitaet, Freiburg, Germany.
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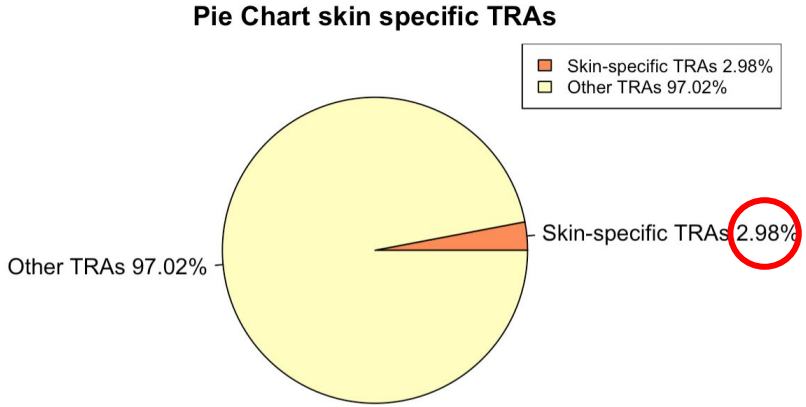
Background

Why do we want to focus on TRAs?

melanoma -> one of the most common type of cancer in humans, also most **metastatic** among all cancers!

tissue restricted antigens (TRAs)

- upregulated in cancer cells for unknown reasons
- potential drug targets



Linares, M. A., Zakaria, A., & Nizran, P. (2015). Skin Cancer. Primary care, 42(4), 645–659. https://doi.org/10.1016/j.pop.2015.07.006
Potrony, M., Badenas, C., Aguilera, P., Puig-Butille, J. A., Carrera, C., Malvehy, J., Puig, S. (2015). Update in genetic susceptibility in melanoma. Annals of translational medicine, 3(15), 210. https://doi.org/10.3978/j.issn.2305-5839.2015.08.11
Smith, R. A., Mettlin, C. J., Eyre, H. (2003). Melanoma and Nonmelanoma Skin Cancer, Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; https://www.ncbi.nlm.nih.gov/books/NBK13764/

What are we working with?
Data Description

Human melanoma cell line with a BRAF-V600D mutation

all microarrays proved to be of good quality.

treated with:

- trametinib: MEK-Inhibitor ⟶ 3h - 24h
- ERK1/2-Inhibitor ⟶ 3h - 24h
- doxycycline-shERK1 ⟶ 3d - 7d

Alqathama A. (2020). BRAF in malignant melanoma progression and metastasis: potentials and challenges. American journal of cancer research, 10(4), 1103–1114.
Kyewski, B., Derbinski, J. (2004) Self-representation in the thymus: an extended view. Nat Rev Immunol 4, 688–698. https://doi.org/10.1038/nri1436
Rosenberg S. A. (1999). A new era for cancer immunotherapy based on the genes that encode cancer antigens. Immunity, 10(3), 281–287. https://doi.org/10.1016/s1074-7613(00)80028-x

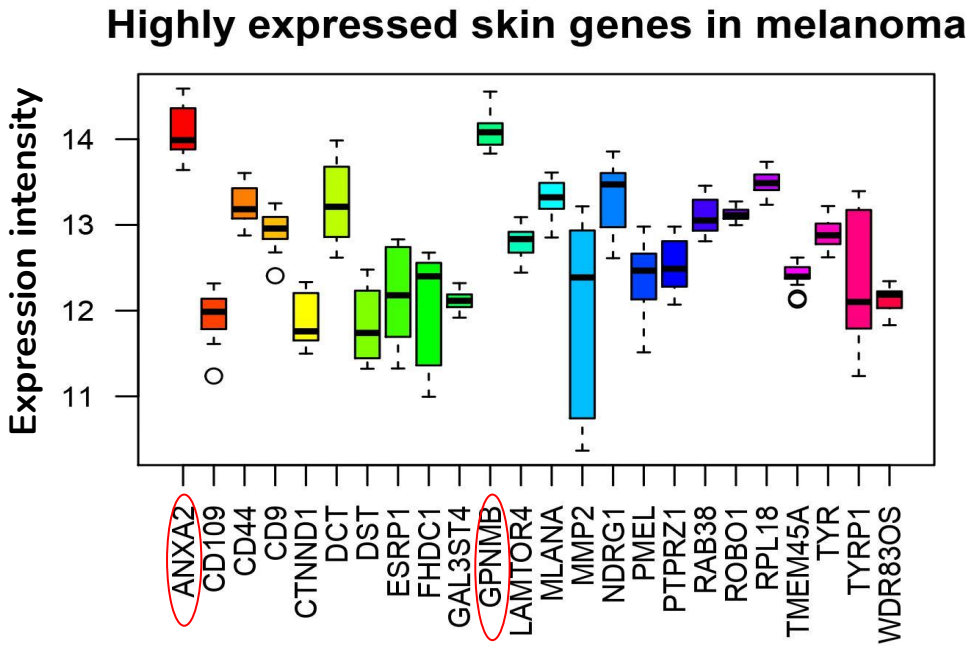
Objectives

Identifying **upregulated skin specific TRAs in skin cancer**, and investigating them to see if they are possible drug targets.

After identifying upregulated genes:

- are our identified genes **related to sun exposure**?
- are there any interesting **groups**?
- are there any **non skin-specific TRAs** that are upregulated in skin cancer?
- do the **efficacy of the drugs** vary?
- can we **predict the expression** of these genes by using other genes (potential targets)?

Highly expressed genes



ANXA2: cancer biomarker
GPNMB: pro-metastatic protein

both responsible for the biogenesis of **melanosomes**!

melanosomes in melanocytes

melanoma develops from **melanocytes**

T., Wang, Z., Niu, R., & Wang, L. (2019). Crucial role of Anxa2 in cancer progression: highlights on its novel regulatory mechanism. Cancer biology & medicine, 16(4), 671–687. https://doi.org/10.20892/j.issn.2095-3941.2019.0228
Setaluri, V., Jayanthy, A. (2013). Coat Color Mutations, Animals, Brenner's Encyclopedia of Genetics, Second Edition, Academic Press, 58-60, ISBN 9780080961569, https://doi.org/10.1016/B978-0-12-374984-0.00275-8.
Delevoye, C., Heiligenstein, X., Ripoll, L., Gilles-Marsens, F., Dennis, M. K., Linares, R. A., Derman, L., Gokhale, A., Morel, E., Faundez, V., Marks, M.S., Raposo, G. (2015). BLOC-1 Brings Together the Actin and Microtubule Cytoskeletons to Generate Recycling Endosomes, Current Biology, Vol 26(1), 1-13, https://doi.org/10.1016/j.cub.2015.11.020

Differential Gene Expression Analysis

Upregulated skin specific TRAs

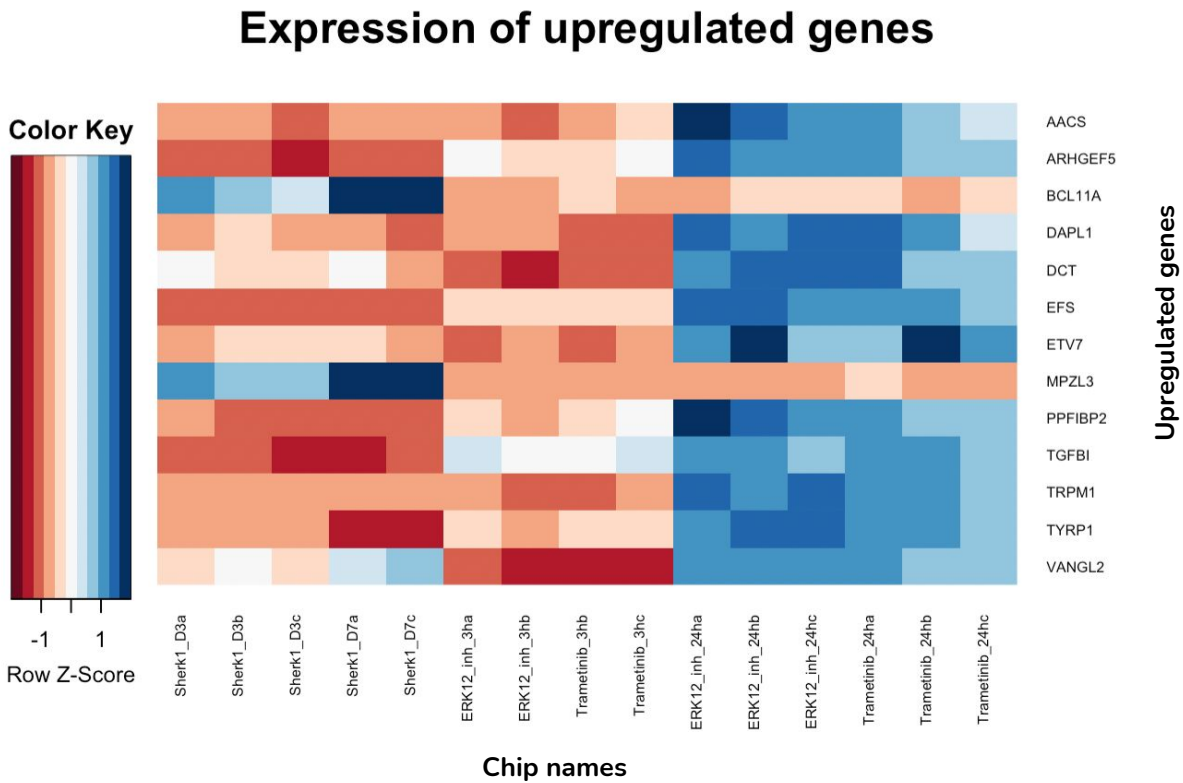
no data available from cells without treatment → start point for differential expression analysis: three hours and three days

- **log2 fold change:** within the chips with the same treatment time-frame
- **t-test:** significance of ten genes from each chip with the highest log-fold change ($p<0.05$)
- **limma analysis**: same genes were found, confirmed

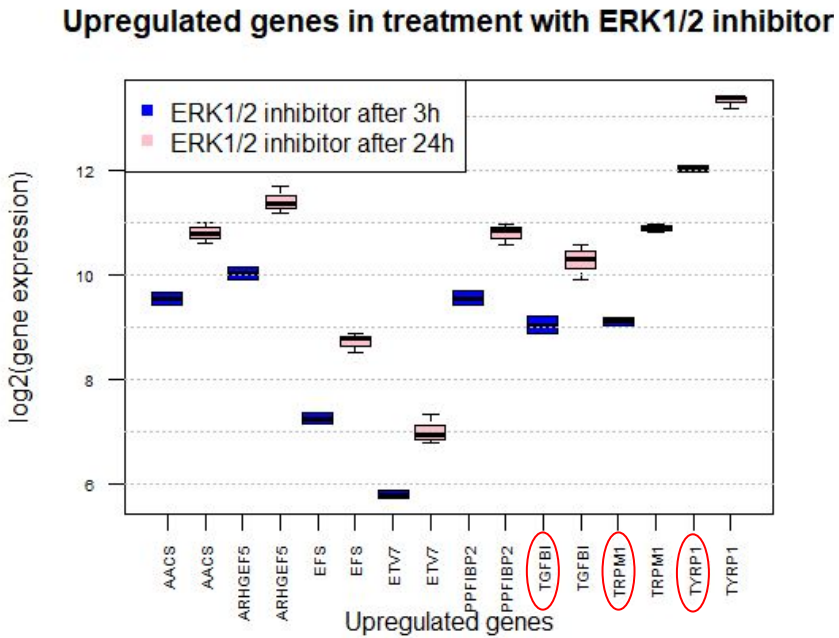
Heatmap of upregulated genes

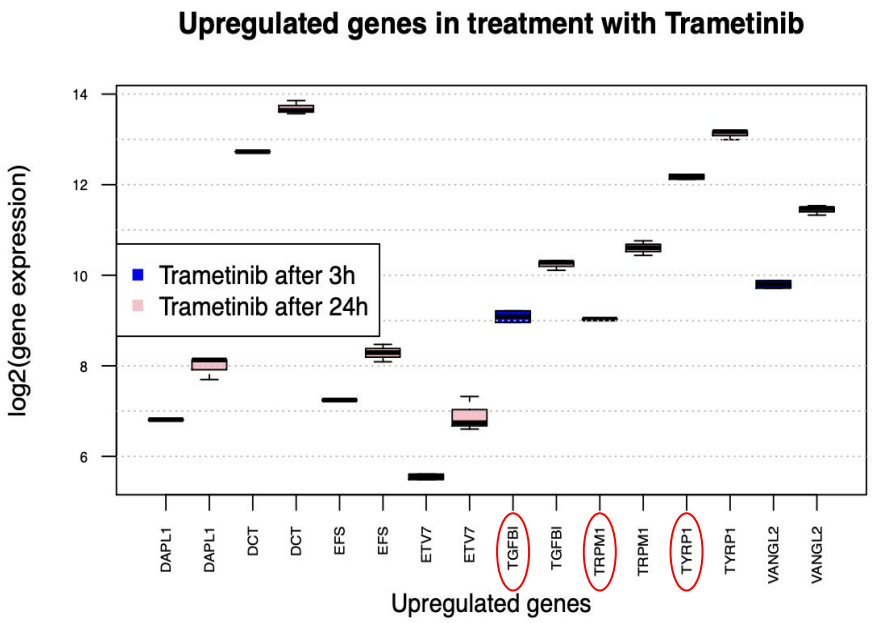
red: lower expression

blue: higher expression



Significantly upregulated skin specific TRAs





Differential gene expression analysis

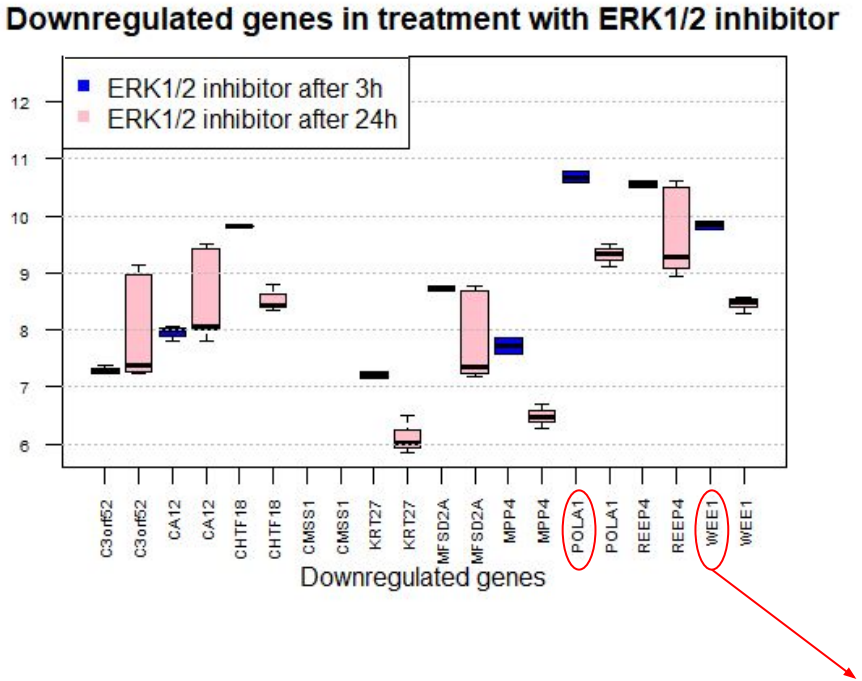
Downregulated skin specific TRAs

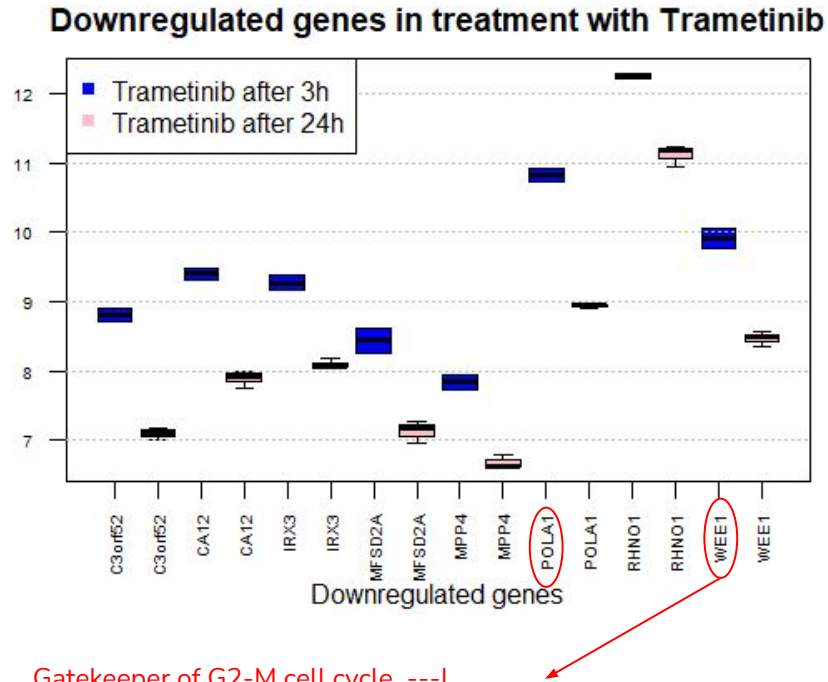
The existence of significantly downregulated genes indicates:

- these genes were probably **inducing tumors before the treatment**
- the **treatment is successfully working.**

Methods: log2 fold change, t-test

Significantly downregulated skin specific TRAs





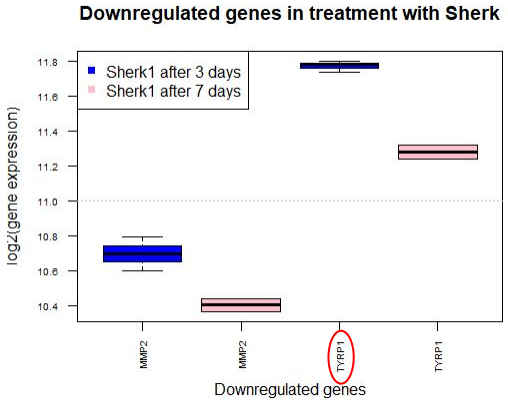
Gatekeeper of G2-M cell cycle ---| proliferation

Bhattacharya, A., Schmitz, U., Wolkenhauer, O., Schönherr, M., Raatz, Y., & Kunz, M. (2013). Regulation of cell cycle checkpoint kinase WEE1 by miR-195 in malignant melanoma. Oncogene, 32(26), 3175–3183. https://doi.org/10.1038/onc.2012.324

Importance of TYRP1 (and MITF!)

- Trametinib & ERK1/2 inhibitor → upregulation
  Dox-shERK → downregulation

- **microphthalmia-associated transcription factor (MITF)**:
  - natural function : melanosome development
  - melanoma : master regulator, crucial for the survival of the melanoma cell



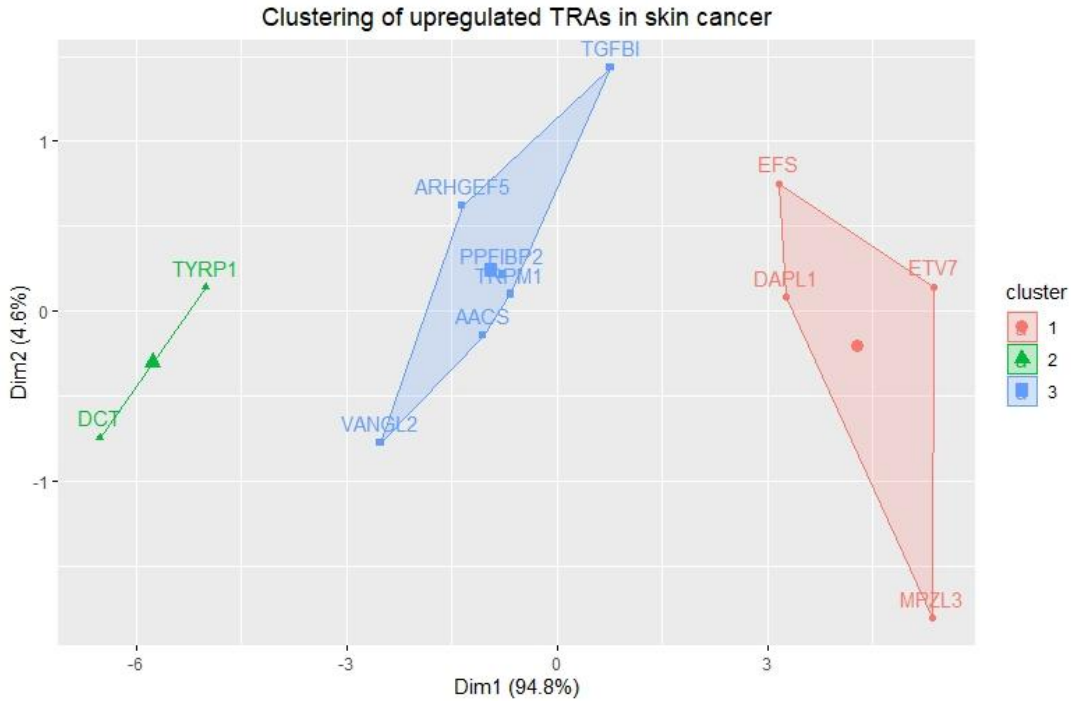
BRAF & MEK inhibitors (trametinib) 2→ MITF → **TYRP1** & melanoma proliferation

1

Rose, A. A., Annis, M. G., Frederick, D. T., Biondini, M., Dong, Z., Kwong, L., Chin, L., Keler, T., Hawthorne, T., Watson, I. R., Flaherty, K. T., Siegel, P. M. (2016). MAPK Pathway Inhibitors Sensitize BRAF-Mutant Melanoma to an Antibody-Drug Conjugate Targeting GPNMB, DOI: 10.1158/1078-0432.CCR-16-1192

Clustering of the upregulated skin-specific TRAs

Visualization via k-means



red: prognostic markers

green: melanin synthesis in the melanosome (TYRP1 and DCT)

blue: MITF and EMT related genes

Zheng, J., et al. 2021. Biomarkers Associated with CD8+ T Cell Infiltration in Childhood AML. https://doi.org/10.21203/rs.3.rs-659529/v1
Zhou, J., et al. 2020. BCL11A Promotes the Progression of Laryngeal Squamous Cell Carcinoma. Frontiers in oncology 10:375. doi:10.3389/fonc.2020.00375
Neumann, L. C., et al. 2011. EFS shows biallelic methylation in uveal melanoma with poor prognosis as well as tissue-specific methylation. BMC Cancer 11:380. doi:10.1186/1471-2407-11-380
Kobayashi, T., Imokawa, G., Bennett, D.C., and Hearing, V.J. (1998). Tyrosinase Stabilization by Tyrp1 (the brown Locus Protein). Journal of Biological Chemistry 273, 31801-31805. https://doi.org/10.1074/jbc.273.48.31801.
Komiya, Y., Onodera, Y., Kuroiwa, M., Nomimura, S., Kubo, Y., Nam, J. M., Kajiwara, K., Nada, S., Oneyama, C., Sabe, H., & Okada, M. (2016). The Rho guanine nucleotide exchange factor ARHGEF5 promotes tumor malignancy via epithelial-mesenchymal transition. Oncogenesis, 5(9), e258. https://doi.org/10.1038/oncsis.2016.59
Noguchi, K., Dalton, A. C., Howley, B. V., McCall, B. J., Yoshida, A., Diehl, J. A., & Howe, P. H. (2017). Interleukin-like EMT inducer regulates partial phenotype switching in MITF-low melanoma cell lines. PloS one, 12(5), e0177830. https://doi.org/10.1371/journal.pone.0177830
Mohammadi, S., Ravindra, V., Gleich, D. F., & Grama, A. (2018). A geometric approach to characterize the functional identity of single cells. Nature communications, 9(1), 1516. https://doi.org/10.1038/s41467-018-03933-2

MITF and EMT in Melanoma

epithelial-mesenchymal transition (EMT): transition from proliferative *epithelial* cells to invasive cells

expectation:

MITF ↑

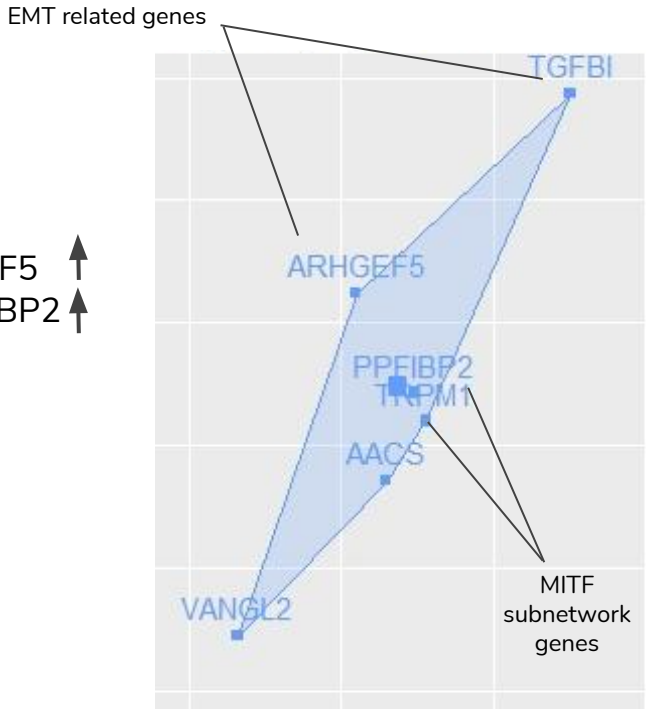
- MITF related genes: TPRM1, PPFBIBP2 ↑
- EMT-like process ❌ : TGFBI, ARHGEF5 ↓
- proliferative stage

MITF ↓

- MITF related genes: TPRM1, PPFBIBP2 ↓
- EMT-like process ✔️ : TGFBI, ARHGEF5 ↑
- invasive stage

our data:

- TGFBI, ARHGEF5 ↑
- TPRM1, PPFBIBP2 ↑



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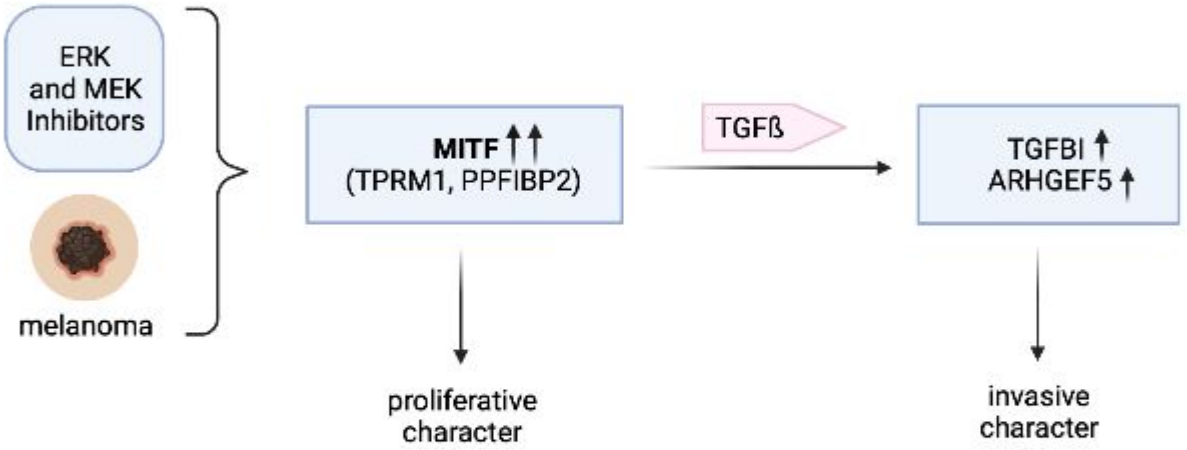
Lavelle, T. J., Alver, T. N., Heintz, K. M., Wernhoff, P., Nygaard, V., Nakken, S., Øy, G. F., Bøe, S. L., Urbanucci, A., & Hovig, E. (2020). Dysregulation of MITF Leads to Transformation in MC1R-Defective Melanocytes. Cancers, 12(7), 1719. https://doi.org/10.3390/cancers12071719
Lauden, L., Siewiera, J., Boukouaci, W., Ramgolam, K., Mourah, S., Lebbe, C., Charron, D., Aoudjit, F., Jabrane-Ferrat, N., Al-Daccak, R. (2014). TGF-β-Induced (TGFBI) Protein in Melanoma: A Signature of High Metastatic Potential, Journal of Investigative Dermatology, Vol:134(6), 1675-1685, ISSN 0022-202X, https://doi.org/10.1038/jid.2014.20
Wellbrock, C., & Arozarena, I. (2015). Microphthalmia-associated transcription factor in melanoma development and MAP-kinase pathway targeted therapy. Pigment cell & melanoma research, 29(4), 390–406. https://doi.org/10.1111/pcmr.12370

Why?



Hypothesis: **high MITF expression** has a responsibility over **proliferation AND metastasis in melanoma.**

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Expression with and without the sun

Genes upregulated under sun exposure

Wilcoxon signed rank test and clustering: significant difference between expressions

ARHGEF5, TGFBI, DAPL1: upregulated by MITF

→ **conformation:** triggering effects of MITF and sun exposure on melanoma

→ upregulation by MITF under sun exposure?

ETV7: usually downregulated in melanoma

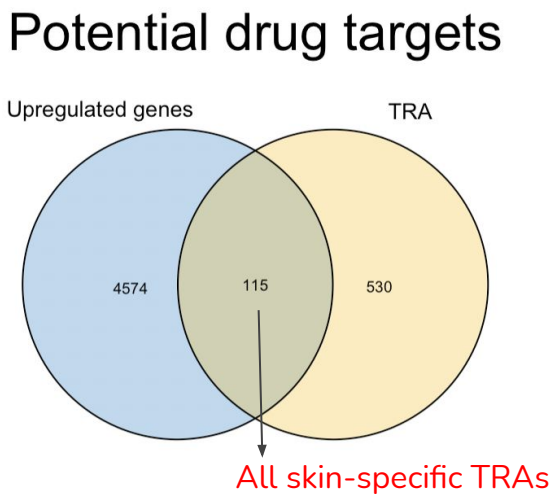
→ upregulated under trametinib under the sun

→ **suspicion:** natural response plus treatment, attacking the tumor microenvironment

Non-skin specific TRAs

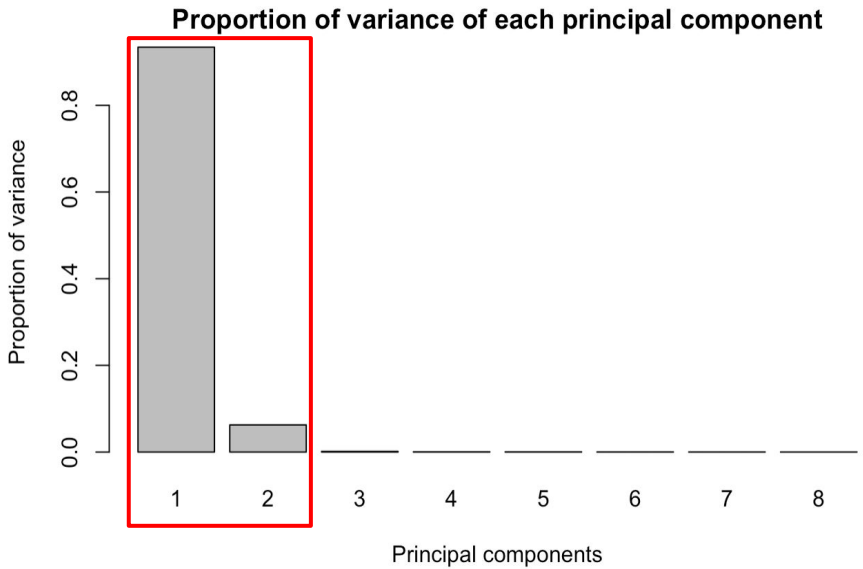
Venn diagram

non skin-specific TRAs that are **upregulated** in skin cancer to be used as potential drug targets.



Principal Component Analysis (PCA)

PC1 and PC2 represent **97.7% of all the variance** of the melanoma dataset.



5 genes with most contribution from each PCs:

- **from PC1:** ANXA2, DCT, NDRG1, TYRP1, PTPRZ1
- **from PC2:** DSP, GJA1, PPP1R14C, TGFBI, UCN2

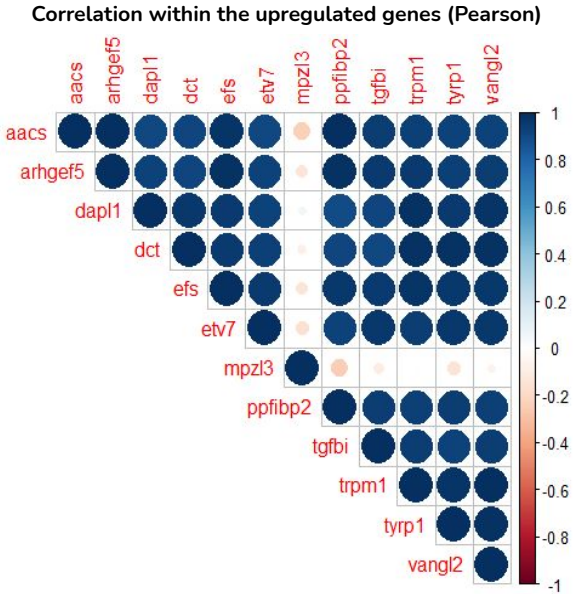
Multiple linear regression

Any significant dependencies between the PC genes and the upregulated genes?

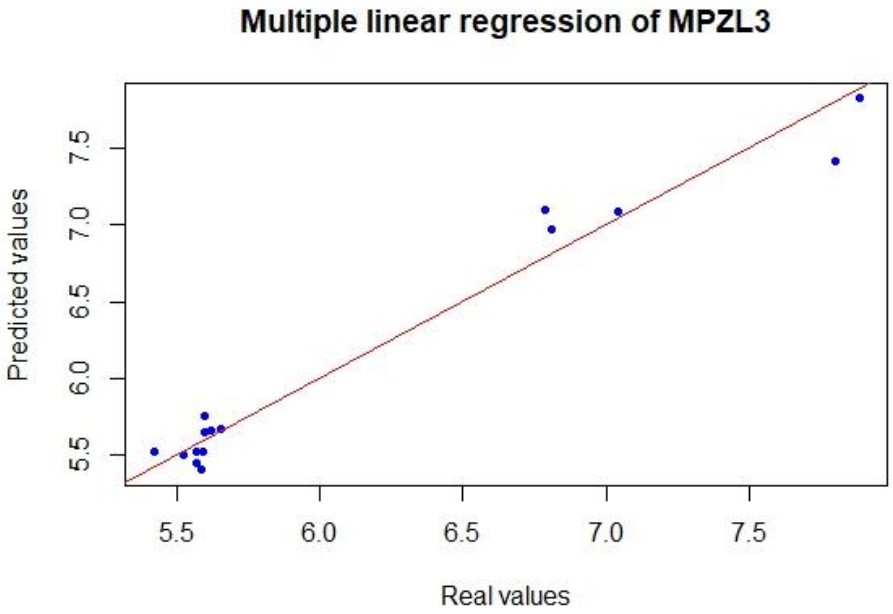
- Residuals meet the conditions!

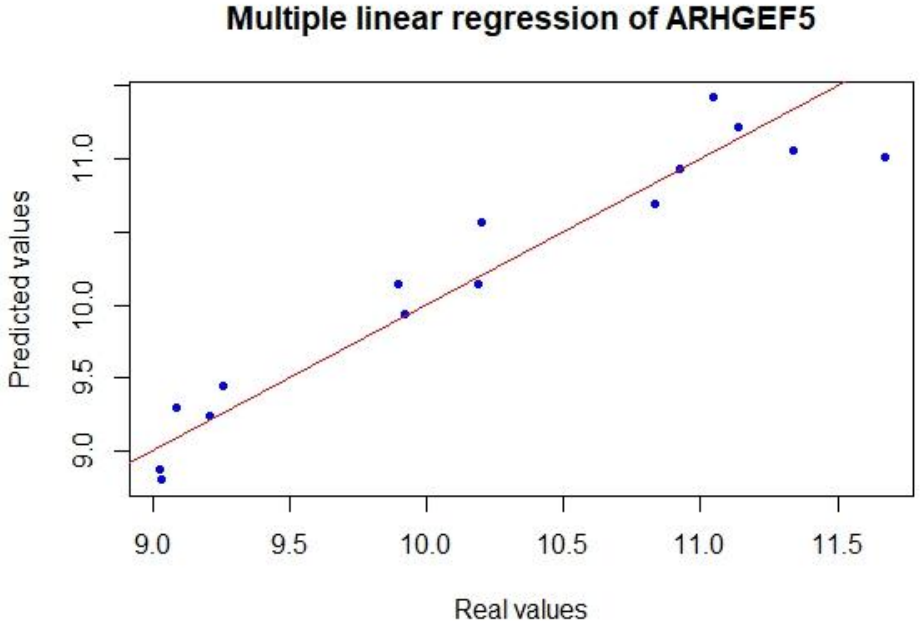
Correlation:

- Upregulated gene representatives: MPZL3, ARHGEF5 (for all the other genes)
- PC gene representatives: GJA1, DSP, ANXA2



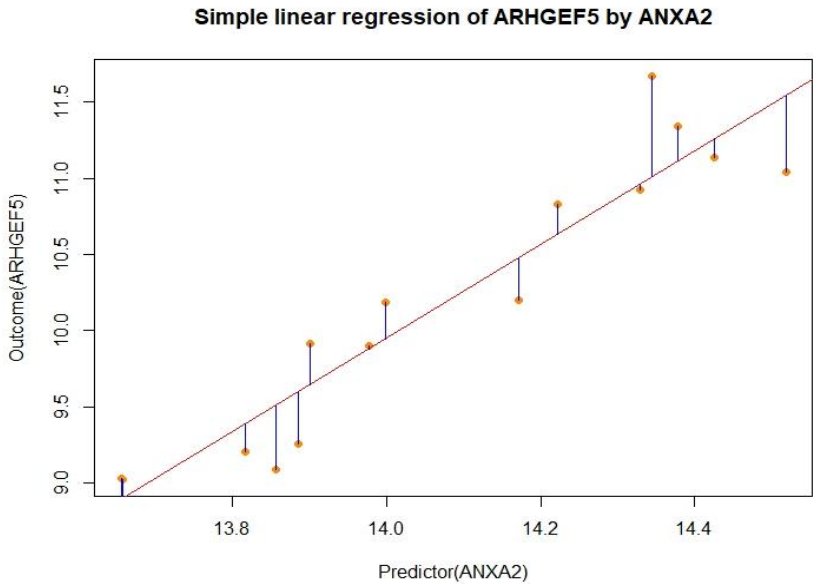
Multiple linear regression





Simple linear regression

Independent variables with the highest impact; ANXA2



high correlation between upregulated genes:

→ almost all upregulated genes predicted together

→ better diagnosis

→ when biological connection: pc genes also drug targets

Wang, T., Wang, Z., Niu, R., & Wang, L. (2019). Crucial role of Anxa2 in cancer progression: highlights on its novel regulatory mechanism. Cancer biology & medicine, 16(4), 671–687. https://doi.org/10.20892/j.issn.2095-3941.2019.0228

Objectives

Upregulated skin specific TRAs in skin cancer identified ✔

Are our identified genes **related to sun exposure**? ✔

- Yes! Four genes that are upregulated are also sun exposed.

Are there any **non skin-specific TRAs** that are upregulated in skin cancer? ✔

- No!

Can we **predict the expression** of the upregulated genes by using other genes (potential targets)? ✔

- Yes! Most of the identified upregulated genes can be predicted by ANXA2, DSP, GJA1.

Do the **efficacy of the drugs** vary? ✔

- Yes! The treatments showed varying effects on different genes. The effect of the MAPK pathway inhibitors on melanoma are questionable, combination therapy may be needed.

Are there any interesting **groups**? ✔

- Yes! MITF related genes have an important role in melanoma and are good drug targets.