# The role of skin specific genes in skin cancer

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Tutor: Nils Mechtel
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.
Dinkelacker, 2019. Chromosomal clustering of tissue restricted antigens, Dissertation, University Heidelberg, Germany.

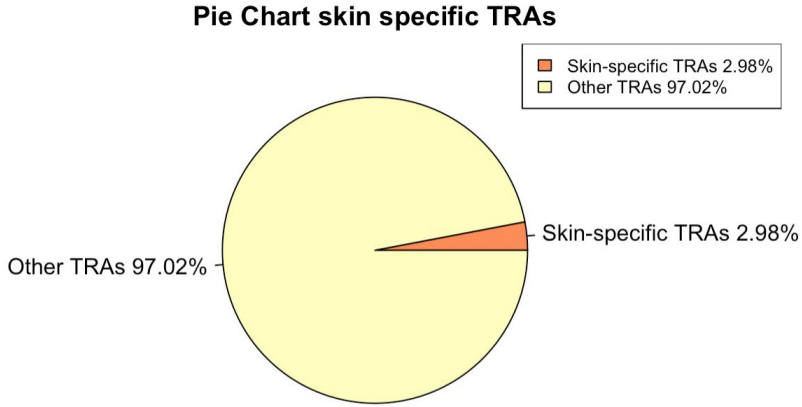
# Background

## 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

WM266-4 human melanoma cell line with a BRAF-V600D mutation

extracted mRNA from cells ->->-> labeled fragmented cRNA, hybridized on microarrays

all microarrays proved to be 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

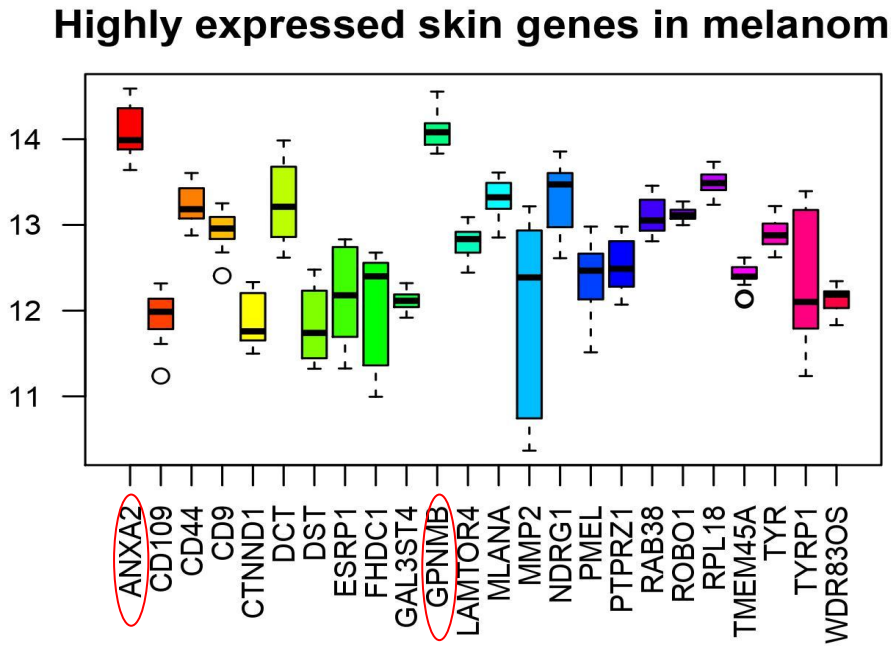
# Objectives

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

# Highly expressed genes




ANXA2: cancer biomarker
GPNMB: pro-metastatic protein

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

melanin production

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

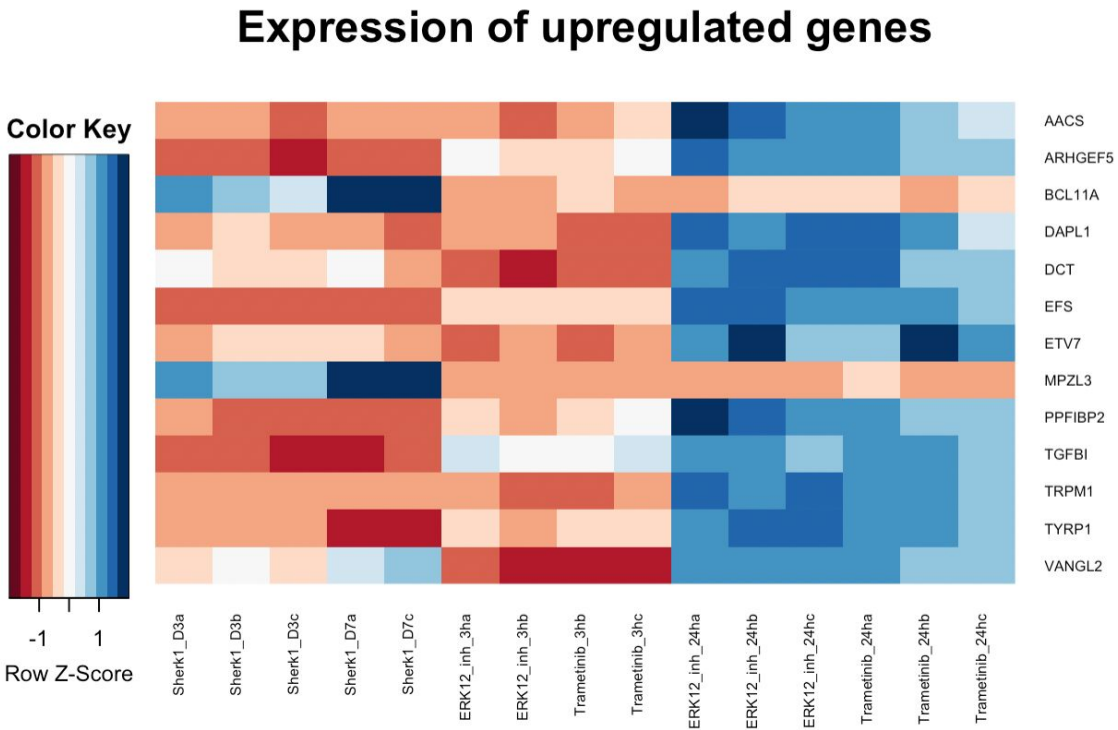
# Differential Gene Expression Analysis

## Upregulated skin specific TRAs

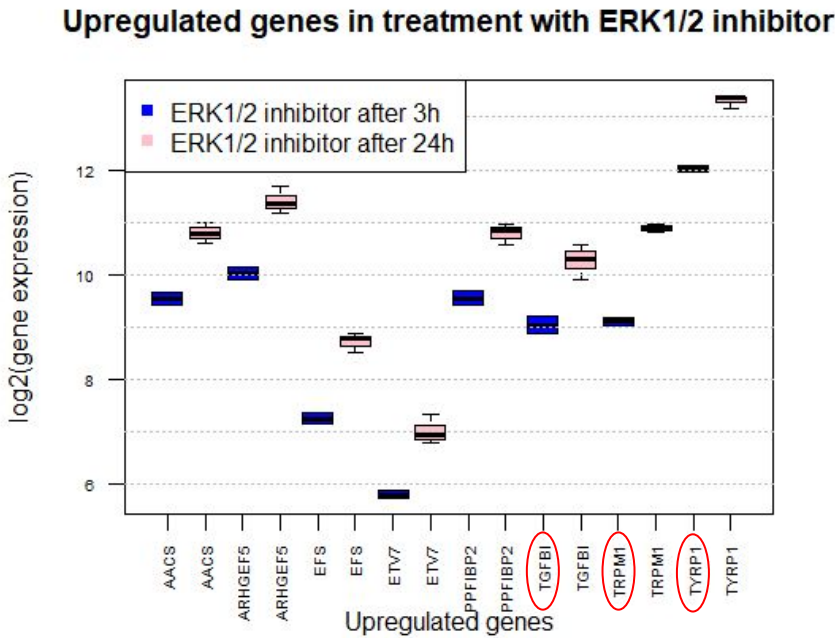
**no data available from cells without treatment** → start point for differential expression analysis: three hours and three days

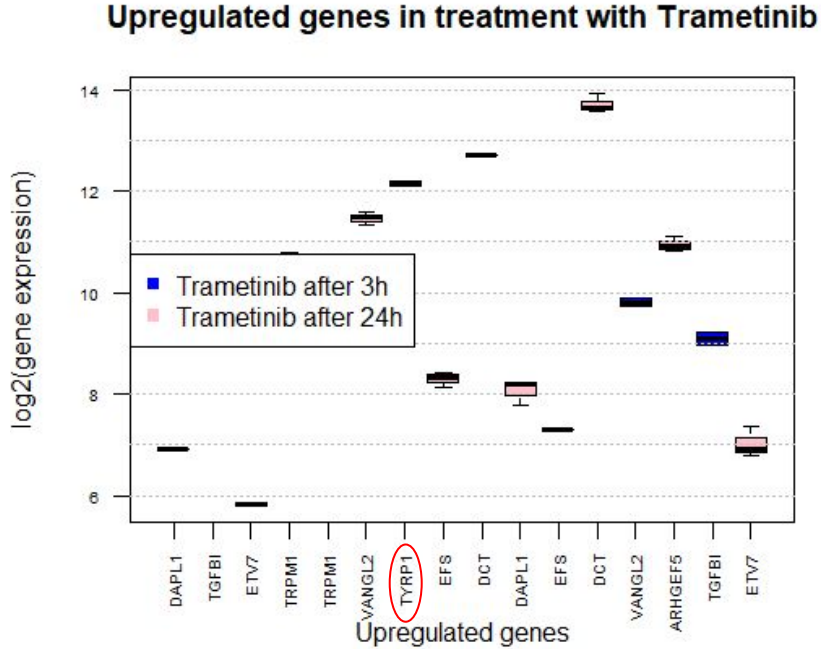
- **log2 fold change:** within the chips with the same treatment time-frame
- **t-test:** significance of ten genes from each chip with the highest log-fold change
- **limma analysis**: same genes were found, confirmed

# Heatmap of upregulated genes



# Significantly upregulated skin specific TRAs



# Differential gene expression analysis

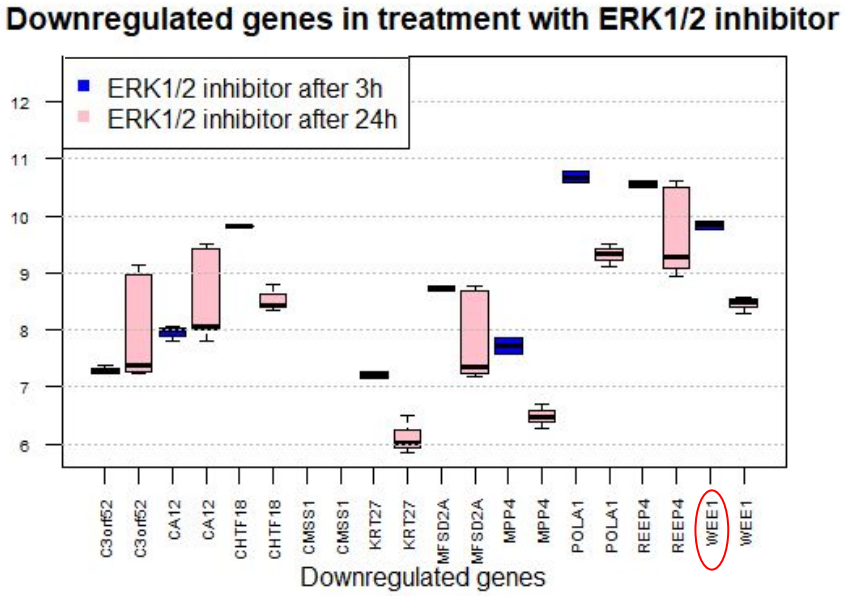
## Downregulated skin specific TRAs

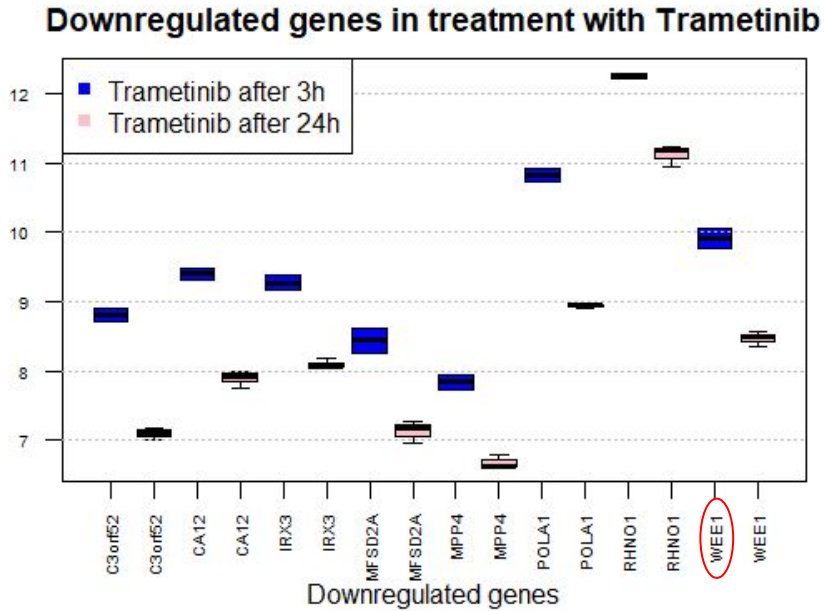
**Methods**: log2 fold change, t-test

The existence of significantly downregulated genes indicates:

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

# Significantly downregulated skin specific TRAs

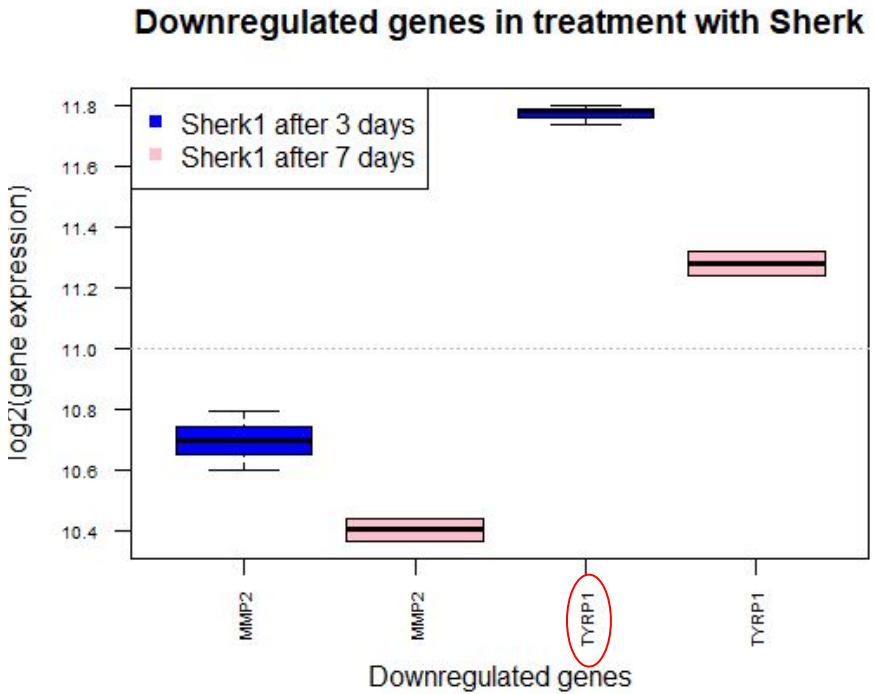





Bhattacharya, A., Schmitz, U., Wolkenhauer, O., Schönherr, M., Raatz, Y., & Kunz, M. (2013). Regulation of cell cycle checkpoint kinase WEE1 by miR-195 in malignant melanoma. Oncogene, 32(26), 3175–3183. https://doi.org/10.1038/onc.2012.324

# Significantly downregulated skin specific TRAs



# The importance of TYRP1

- significantly **upregulated under Trametinib** treatment, but **significantly downregulated under dox-shERK1.**
- **relation to MITF: upregulation under BRAF and MEK inhibitors**, in this case Trametinib
- microphthalmia-associated transcription factor (MITF):

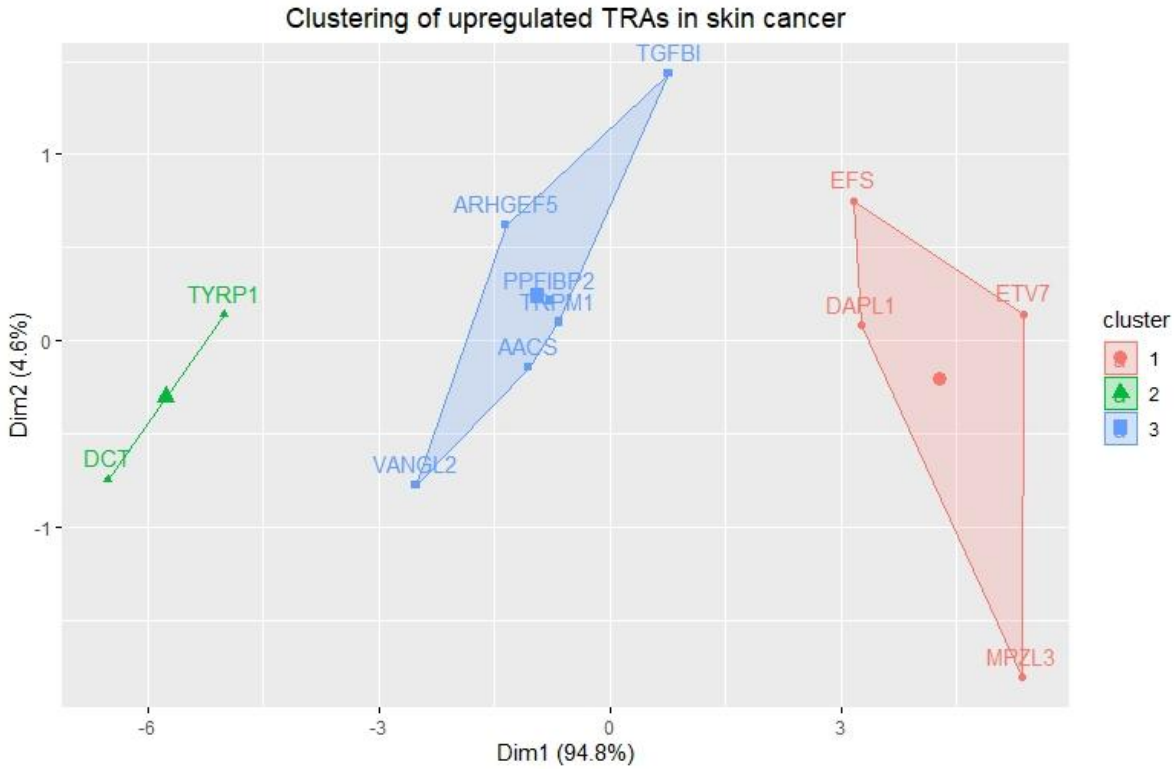
  natural function → pigmentation

  in melanoma: master regulator, crucial for the maintenance and survival of the melanoma cell



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




**red:** prognostic markers

**green:** melanosome biosynthesis (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 ↑

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

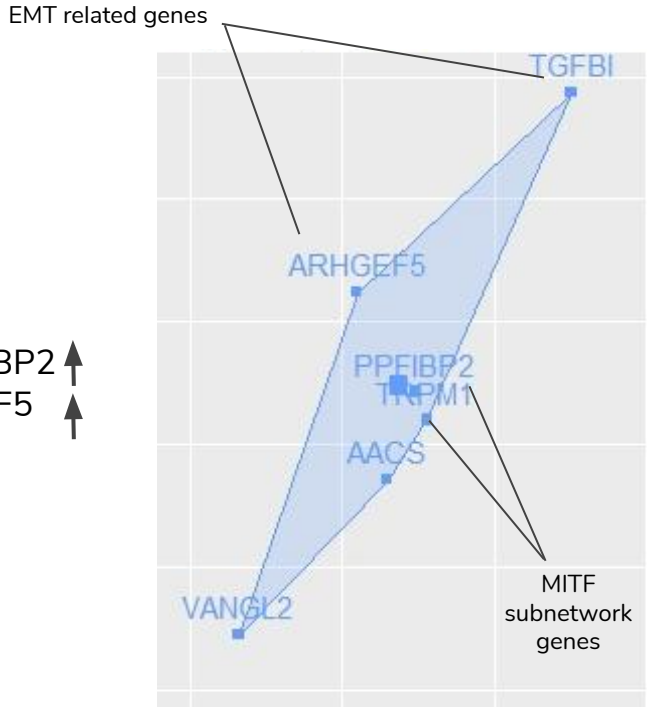
MITF ↓

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

**our data:**

MITF ↑

- TPRM1, PPFBIBP2 ↑
- TGFBI, ARHGEF5 ↑





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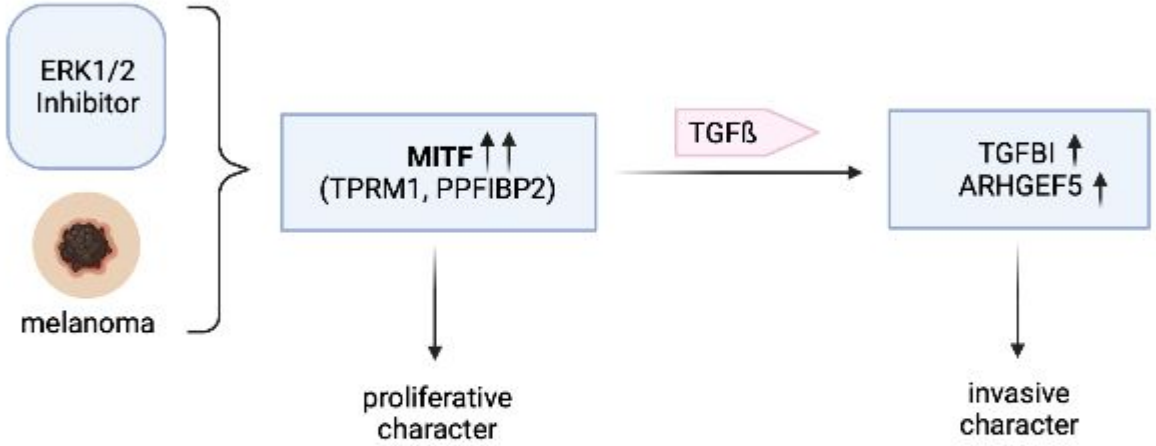
Lavelle, T. J., Alver, T. N., Heintz, K. M., Wernhoff, P., Nygaard, V., Nakken, S., Øy, G. F., Bøe, S. L., Urbanucci, A., & Hovig, E. (2020). Dysregulation of MITF Leads to Transformation in MC1R-Defective Melanocytes. Cancers, 12(7), 1719. https://doi.org/10.3390/cancers12071719
Lauden, L., Siewiera, 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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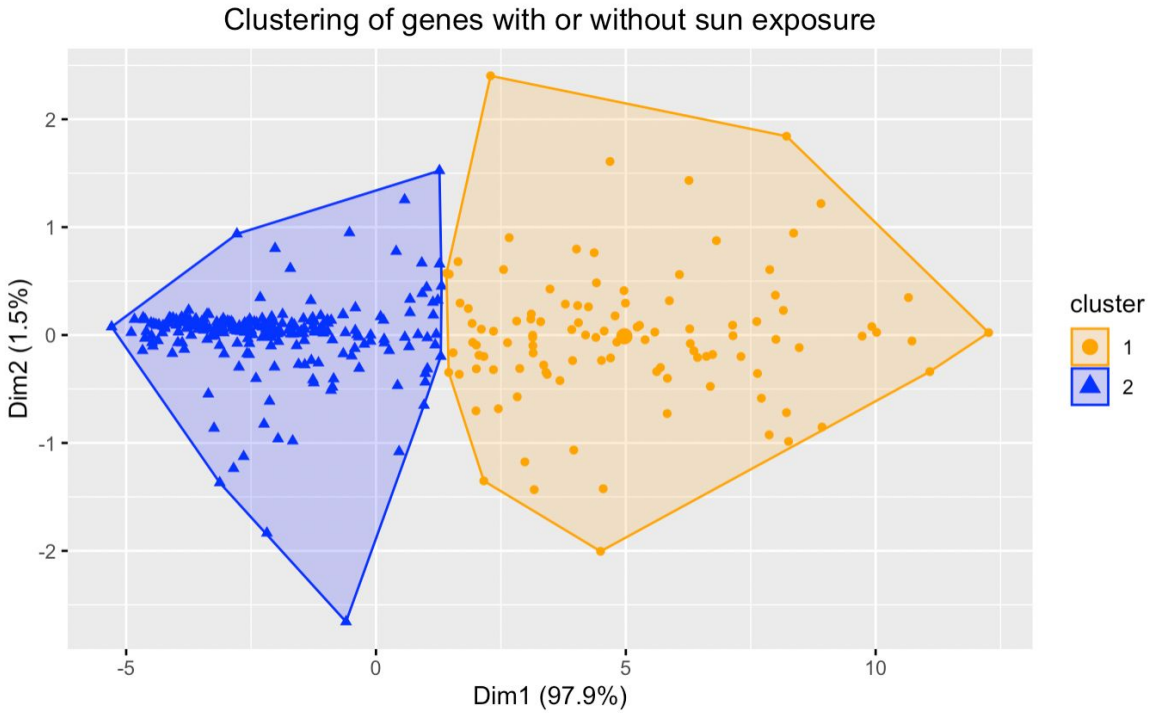
# Expression with and without the sun

## Visualization via k-means




Wilcoxon signed rank test: significant difference between the two

orange: sun exposure
blue: no sun exposure

# Genes upregulated under sun exposure

ARHGEF5 and TGFBI: allow a high metastatic potential, upregulated by MITF

DAPL1: also upregulated by MITF

→ MITF upregulating genes under sun exposure

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

ETV7: usually downregulated in melanoma

→ upregulated under trametinib under the sun

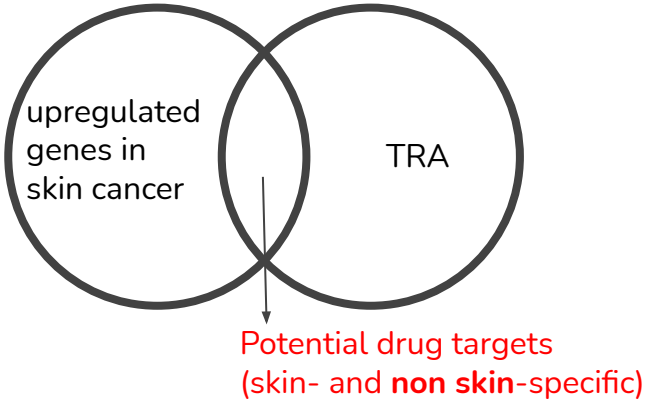
→ **suspicion:** natural response, and treatment, attacking the tumor microenvironment

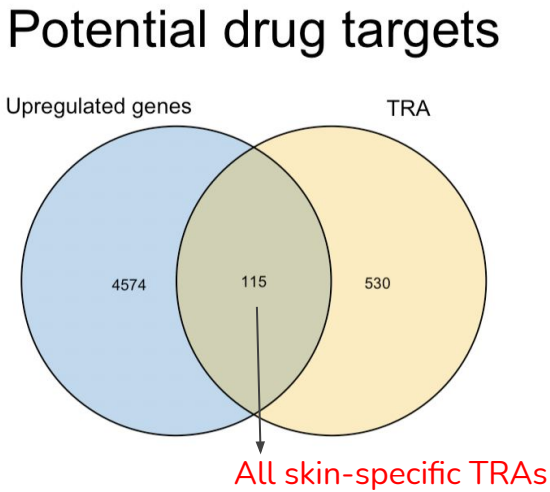
Lauden, L., Siewiera, J., Boukouaci, W., Ramgolam, K., Mourah, S., Lebbe, C., Charron, D., Aoudjit, F., Jabrane-Ferrat, N., Al-Daccak, R. (2014). TGF-β-Induced (TGFBI) Protein in Melanoma: A Signature of High Metastatic Potential. Journal of Investigative Dermatology, Vol:134(6), 1675-1685, ISSN 0022-202X, https://doi.org/10.1038/jid.2014.20.
Kuroiwa, M., Oneyama, C., Nada, S., & Okada, M. (2011). The guanine nucleotide exchange factor Arhgef5 plays crucial roles in Src-induced podosome formation. Journal of cell science, 124(Pt 10), 1726–1738. https://doi.org/10.1242/jcs.080291
Qu, H., Zhao, H., Zhang, X., Liu, Y., Li, F., Sun, L., & Song, Z. (2020). Integrated Analysis of the ETS Family in Melanoma Reveals a Regulatory Role of ETV7 in the Immune Microenvironment. Frontiers in immunology, 11, 612784. https://doi.org/10.3389/fimmu.2020.612784
Ma, X., Hua, J., Zheng, G., Li, F., Rao, C., Li, H., Wang, J., Pan, L., & Hou, L. (2018). Regulation of cell proliferation in the retinal pigment epithelium: Differential regulation of the death-associated protein like-1 DAPL1 by alternative MITF splice forms. Pigment cell & melanoma research, 31(3), 411–422. https://doi.org/10.1111/pcmr.12676

# Non-skin specific TRAs

## Venn diagram

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

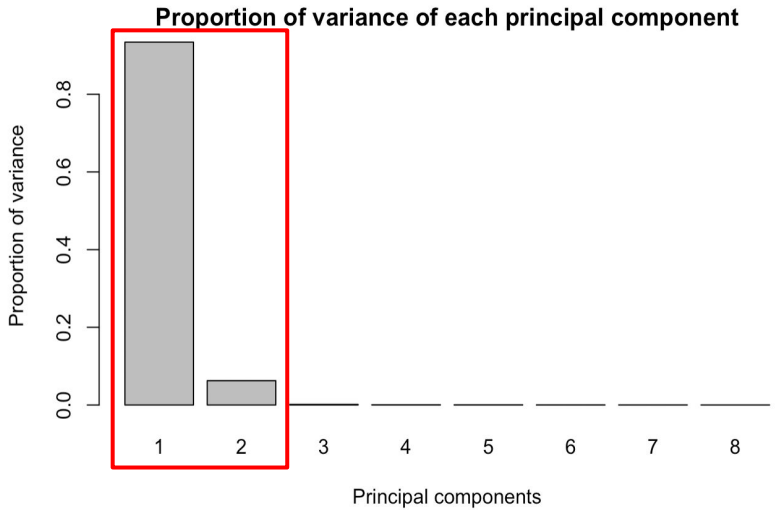


# Principal Component Analysis (PCA)

PCA is carried out only with the second time points (**24h and 7d**).

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




5 genes with the top variance from each components:

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

# Multiple linear regression

Is there any significant dependencies between the PC genes and the upregulated genes?

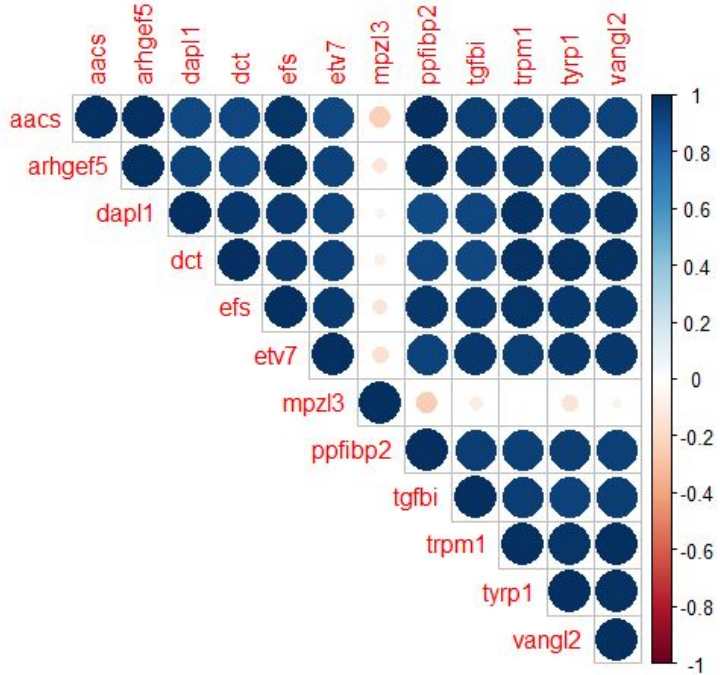
- prediction of the expressions
- PC genes → better prognosis

But first:

- do residuals have a mean of zero?
- do they correlate within themselves or with the predictors?

# Multiple linear regression; Correlation

**Correlation within the upregulated genes (Pearson)**



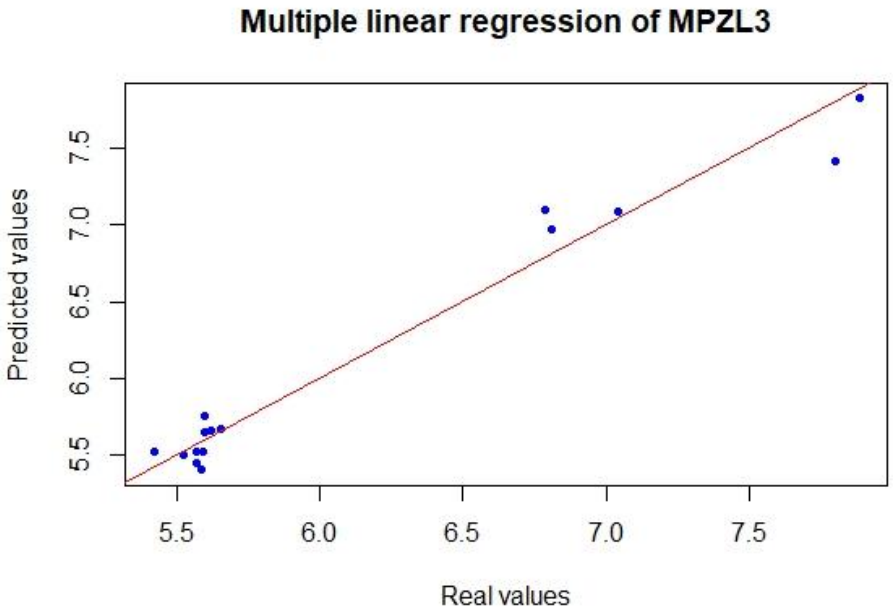

upregulated gene representatives:

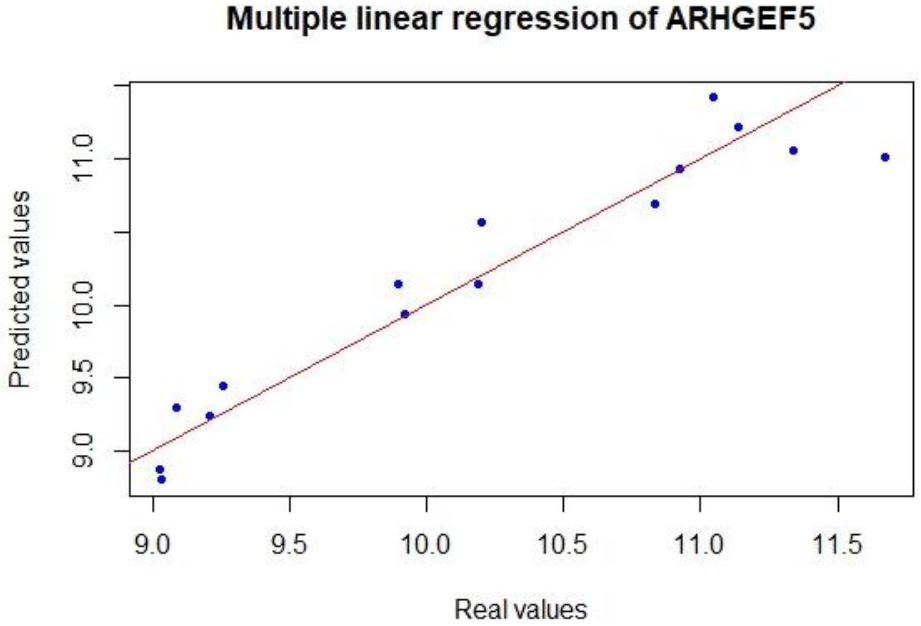
- MPZL3
- ARHGEF5 (for all the other genes)

PC gene representatives:

- GJA1
- DSP
- ANXA2

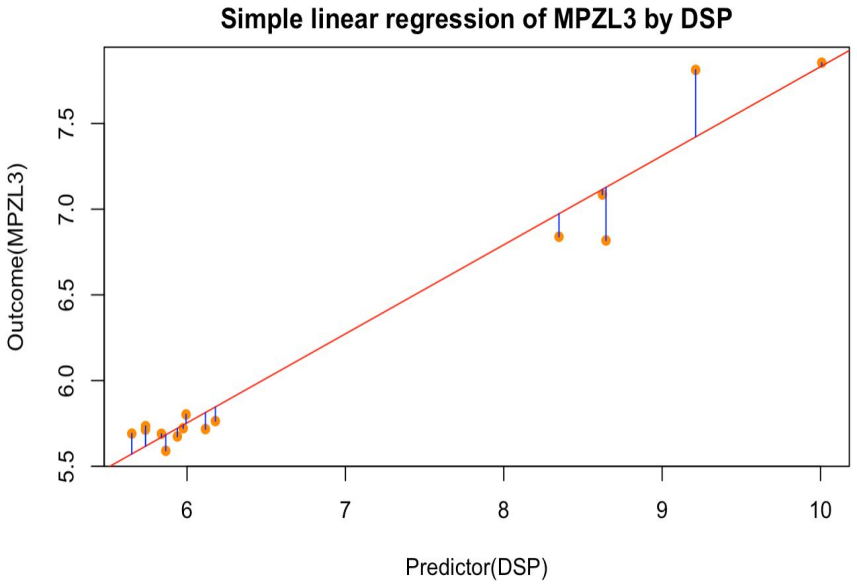
# Multiple linear regression



# Simple linear regression

## Independent variables with the highest impact; DSP




DSP has the highest impact on the MPZL3 model.

DSP: desmosomal protein

- escaping the immune system
- desmosomal proteins are used to identify melanomas

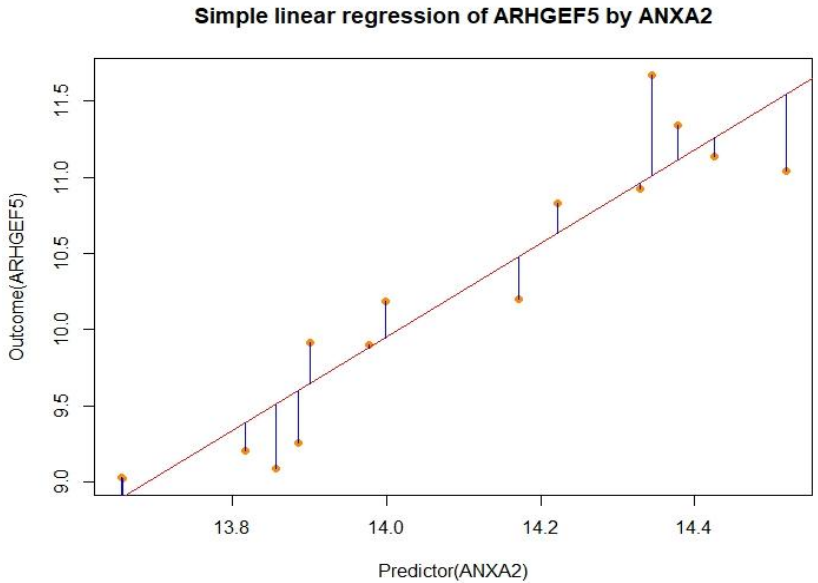
MPZL3

- is related to microsatellite instability
- doesn't correlate with the rest; unknown; further research

Salerno, E. P., Bedognetti, D., Mauldin, I. S., Deacon, D. H., Shea, S. M., Pinczewski, J., Obeid, J. M., 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

# Simple linear regression

Independent variables with the highest impact; ANXA2




- high correlation of upregulated genes: almost all upregulated genes predicted together
- ANXA2 already popular drug target in cancer



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!

Are there any interesting **groups**? ✔

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

Can we **predict the expression** of these 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.