**FINAL REPORT TEMPLATE**

**1. FINAL PUBLISHABLE SUMMARY REPORT**

EPI-PATHO-STEM project aimed to study epithelial stem cell location, dynamics and molecular pathways in heath and pathology. As a model, we studied the stratified epithelium of the skin (epidermis) and the cornea. These closely related tissues which line the external surface of the mammalian body and eye are multi-layered tightly packed tissues providing protection against infections, injuries and water loss1–4. However, the cornea is a specialized transparent tissue that plays an optic role. Interestingly, the cornea becomes skin-like or may even grow hair in mice lacking *DKK2*5 or *NOTCH1*6genes and in human pathology. Yet, the question of why corneal transparency is lost in various diseases is poorly understood. The accomplish the aims of EPI-PATHO-STEM, we used genetic mouse models for labelling and tracking the origin of stem/progenitor cells in living animals (articles 1-2 below), study the regulation of stem/progenitor cells extracted from cadaveric cornea by SOX2 gene (article 3), we have generated mouse strains that lack or over-express MIR184 gene and define the role of this gene in the epidermis (article 4), and finally, we discovered a new role for RAS genes in pluripotent stem cells (article 5).

A better understanding of epithelial stem cell fate decisions is essential for isolating them and optimizing their expansion ex vivo before transplanting them back onto skin burn patients and blind patients. Unfortunately, it is not possible yet to purify epithelial stem cells and their expansion ex vivo is associated with a rapid loss of stemness and multipotency. Consequently, skin appendages are not formed in such grafts. Therefore, the molecular cues that underlie stem cell fate decisions need to be unravelled.

We believe that EPI-PATHO-STEM addressed major questions in the field of stem cell biology revealed new signalling pathways that modulate stem cell self-renewal and differentiation and will bring insightful information of high clinical relevance to patients that suffer from stem cells deficiency diseases or diseases that involve imbalance between proliferation and differentiation. Among these pathologies are aniridia, ectodermal dysplasia, psoriasis, cancer and more. As detailed below, our findings shed light on major questions in the field and may lead to novel therapy in follow up studies. A summary of our research interest and of EPI-PATHO-STEM research is described here below and on our website <https://shalomfe.net.technion.ac.il/>.

**(Article 1) Lineage tracing of stem and progenitor cells of the murine corneal epithelium**7,8: Accumulating evidence supports the dogma that the corneal epithelium is regenerated by stem cells located exclusively in the limbal niche, at the corneal periphery. Accordingly, limbal stem cells (LSCs) give rise to progenitors that proliferate and migrate centripetally to repopulate the corneal epithelium, which has a short turnover. Moreover, LSC loss leads to corneal opacity and blindness while limbal grafting restores patients' vision. However, contradicting data suggested that the limbus does not participate in corneal homeostasis and that the cornea contains stem cells.

To address this burning question, we performed lineage tracing experiments using R26R-Confetti mice to follow K14+ limbal/corneal epithelial cells stochastically induced to express one out of four fluorescent genes. In homeostasis, radial limbal stripes of slow migrating cells proceeded towards the corneal center while, infrequently, slow cycling limbal clones resembling quiescent stem cells were observed. Additionally, rare corneal clones that did not migrate centripetally, but survived for over 4 months, were inspected. In contrast to limbal stripes, corneal clusters had minor contribution to tissue replenishment in homeostasis. Corneal cells, however, significantly contributed to mild wound repair while large limbal streaks appeared within a week following severe wounding that coincided with partial loss of corneal transparency. Altogether, this study suggests that the limbus plays a major role in corneal renewal, while the cornea has a long-term self-maintenance capability. This mouse model will allow for addressing key questions in corneal stem cell biology in the future.

**(Article 2) Corneal committed cells restore the stem cell pool and tissue boundary following injury (submitted to publication).** During morphogenesis, it is essential to preserve segregated cellular compartments to properly regulate cell fate decisions. While embryonic tissues possess extremely high plasticity and ability for tissue repair, the question of whether and how adult tissues cope with acute SC loss or boundary disruption has remained open.

The cornea serves as an excellent model for studying tissue bordering and SC biology. We discovered that K15-GFP transgene labels the murine corneal epithelial boundary and SC niche known as the limbus. K15-GFP+ basal epithelial cells expressed SC markers and were located at the margin site of corneal regeneration, as evident by lineage tracing. Remarkably, surgical deletion of the SC pool of the limbus was restored by corneal committed cells which underwent dedifferentiation into bona fide SCs. Notably, the recovered corneas displayed normal marker expression and appropriate dynamic of LSC regeneration. Interestingly, however, damage to the limbal stromal niche abolished K15-GFP recovery and led to loss of corneal transparency. We provide direct evidence for pathological wound healing by adjacent conjunctival tissue that was accompanied by neovascularization, loss of transparency and blindness. Altogether, this study indicates that committed corneal cells have large plasticity to dedifferentiate, repopulate the SC pool and correctly reform tissue boundary. By contrast, loss of SC and boundary of the cornea lead to impaired tissue functionality and pathology.

**(Article 3) Sox2 controls stemness, proliferation and epigenetic landscape of corneal epithelial cells (under review).** Sox2 has been extensively studied in the context of pluripotent stem cells. Interestingly, however, a major phenotype of patients that are carriers of point mutations in *SOX2* gene, is eye absence or small eye coupled with corneal neovascularization, loss of corneal transparency and blindness. The latter symptoms is generally linked with stem cell (SC) failure. Yet, the expression pattern and function of Sox2 in the cornea remained unclear.

Here we report that Sox2 is expressed by stem and progenitor but not by differentiated cells of the corneal epithelium. Notably, Sox2+ SCs of the cornea displayed low epigenetic repressive marks while knockdown of Sox2 significantly enhanced this phenotype and reduced SC clonogenic potential. Moreover, Sox2 was required for cell proliferation while its repression resulted in accelerated cell differentiation.

Interestingly, we found that miR-450b which was reciprocally expressed with Sox2 in lens, neural and corneal lineages, is a direct repressor of Sox2. miR-450b repressed Sox2 protein expression, reduced stemness and induced differentiation of corneal epithelial cells. Altogether, we propose that Sox2 controls stemness, epigenetic state and proliferation while its repression by miR-450b induces differentiation. In light of these finding, we propose that Sox2 mutations may lead to SC failure and/or disturb the balance between cell proliferation and differentiation.

**(Article 4) microRNA-184 induces a commitment switch to epidermal differentiation (revised version was submitted).** miR-184 is an extremely highly evolutionary conserved microRNA (miRNA) from fly to human. The importance of miR-184 was underscored by the discovery that point mutations in miR-184 gene lead to corneal/lens blinding disease. However, miR-184-related function in vivo remained unclear. We discovered that miR-184 knockout mouse model displayed increased p63 expression in line with epidermal hyperplasia while forced expression of miR-184 by stem/progenitor cells enhanced Notch pathway and induced epidermal hypoplasia. In line, miR-184 reduced clonogenicity and accelerated differentiation of human epidermal cells. We showed that by directly repressing cytokeratin 15 (K15) and FIH1, miR-184 induces Notch activation and epidermal differentiation. The disease-causing miR-184C57U mutant failed to repress K15 and FIH1 and to induce Notch activation suggesting a loss-of-function mechanism. Altogether, we propose that, by targeting K15 and FIH1, miR-184 regulates the transition from proliferation to early differentiation, while miss-expression or mutation in miR-184 results in impaired homeostasis.

**(5) RAS regulates the transition from naïve to primed pluripotent stem cells (under reviewing).** Cancer cells and stem cells share several common features and signaling pathways. The transition from naïve to primed state of pluripotent stem cells is hallmarked by epithelial to mesenchymal transition, metabolic switch from oxidative phosphorylation to preferential usage of aerobic glycolysis, and dramatic changes in the epigenetic landscape. Since these changes are also hallmarks of neoplastic cell transformation, we hypothesized that oncogenic pathways may be involved in this process.

We discovered that the activity of RAS proteins is repressed in conditions that maintain naïve undifferentiated state of mouse embryonic stem cells (ESCs) and that by contrast, all three RAS isoforms are significantly activated upon early differentiation induced by either LIF withdrawal, embryoid body formation, or transition to the primed state. Forced expression of active RAS was sufficient to induce an exit from naïve state coupled with expression of repressive epigenetic marks and N-CADHERIN in expense of E- CADHERIN. By contrast, inhibition of RAS by short hairpin RNA or by a pharmacological RAS inhibitor, significantly attenuated differentiation. Altogether, this study indicates that RAS is located at a key junction of early ESC differentiation and that it controls key processes in priming of naïve cells.

**2. USE AND DISSEMINATION OF FOREGROUND**

**Section A (public) – DISSEMINATION MEASURES**

**Dissemination activities:**

**The results of EPI-PATHO-STEM project were presented the in international and national scientific meeting:**

**INTERNATIONAL CONFERENCES**

* Gordon Research Conference on Cornea, Biology & Pathobiology; Ventura CA, USA; Feb 28- Mar 4, 2016. Title: The origin, fate and regulation of stem cells in the corneal epithelium.
* ISSCR 14th Annual Meeting on Stem Cell Research; Stockholm, Sweden; Jun 24-27 2015. Title: The origin and regulation of epithelial stem cells of the cornea.
* The Bowman Club 16th Annual Meeting on Eye Research; Liverpool, UK; March 21st 2014. Title: The promises of pluripotent stem cells: cell therapy, gene therapy & modeling congenital diseases.
* ARVO 2014 Meeting on Vision and Ophthalmology Research; Orlando FL, USA; May 4-8 2014. Title: miR-184 and miR-450b are essential regulators of corneal epithelial fate.
* Gordon Research Conference on Epithelial Keratiniztion; Ciocco, Italy; May 12-17 2013. Title: miR-184 represses K15 and induces epithelial stem cell differentiation.

**NATIONAL CONFERENCES**

* Swiss Institute of Developmental Biology symposium:Development of sensory systems, July 5th 2017. Title: Corneal stem cell niche and dynamics in health and disease.
* Israel Society for Vision & Eye Research 37th annual meeting; Kfar Hamaccabiah, Israel; March 15-16 2017. Session moderator: Animal models.
* Israel Society for Vision & Eye Research 36th annual meeting; Kfar Hamaccabiah, Israel; April 11-12 2016. Session moderator: Animal models.
* The 6th ISCS Israel International Stem Cell Meeting; Tel Aviv, Israel; Apr 5-6 2016. Title: New mechanisms of skin/corneal diseases: a lesson from pluripotent stems cells.
* TIDSB meeting, Weizmann Institute, 2015, Oct 13th; Title: Stem cell niche of a transparent tissue: revisiting the old dogma
* The 2nd BIRAX UK-Israel Regenerative Medicine Conference; Haifa, Israel; Mar 25th 2014. Title: Skin and corneal therapies using pluripotent stem cells.
* Israel Society for Vision & Eye Research 37th annual meeting; Airport city, Israel; Mar 15-16 2017. Title: Corneal stem cell niche and dynamics in health and disease.
* Technion/Kyoto symposium on pluripotent stem cells and regenerative medicine; Technion, Israel; Mar 14 2017; Title: modeling skin and corneal pathophysiology with pluripotent stem cells.
* Israel Society for Vision & Eye Research 36th annual meeting; Airport city, Israel; Apr 11-12 2016. Title: Lineage tracing of stem/progenitor cells of the murine corneal epithelium.
* Israel Society for Vision & Eye Research 33th annual meeting; Airport city, Israel; Mar 15-16 2013. Title: Study of corneal epithelial pathophysiology and therapy by induced pluripotent stem cells.
* Frontiers in Biomedical Science, Nazareth, Israel; Feb 25-26 2014. Title: Study of skin and corneal stem cells and pathophysiology.
* The 5th ISCS International Stem Cell Meeting; Jerusalem, Israel; Oct 8-9 2013. Title: Pluripotent stem cell model for studying skin and corneal pathophysiology and therapy.

**Publications**

The results of EPI-PATHO-STEM project were translated into scientific publications in respected journals. Below is a list of published articles and an additional list of articles that are in the process of publication.

**Published articles:**

1. Allele-specific silencing of EEC p63 mutant R304W restores p63 transcriptional activity. Melino G., Novelli F, Lena AM, Panatta E, Nasser W, Shalom-Feuerstein R, and Candi E. **Cell Death & Disease 2016.** May 19;7.
2. A Method for lineage tracing of corneal cells using multi-color fluorescent reporter mice. Amitai‐Lange A, Berkowitz E, Altshuler A, Dbayat N, Suss-Toby E, Tiosano B, Shalom‐Feuerstein R. **JoVE, 2015** Dec 18;(106).
3. [Interactions of Melanoma Cells with Distal Keratinocytes Trigger Metastasis via Notch Signaling Inhibition of MITF.](http://www.ncbi.nlm.nih.gov/pubmed/26236014) Golan T, Messer AR, Amitai-Lange A, Melamed Z, Ohana R, Bell RE, Kapitansky O, Lerman G, Greenberger S, Khaled M, Amar N, Albrengues J, Gaggioli C, Gonen P, Tabach Y, Sprinzak D, Shalom-Feuerstein R, Levy C. **Mol Cell. 2015** Aug 20;59(4):664-76.
4. Amitai-Lange A, Altshuler A, Bubley J, Dbayat N, Tiosano B, Shalom-Feuerstein R. Lineage tracing of stem and progenitor cells of the murine corneal epithelium. **Stem Cells 2015** Jan;33(1):230-9.
5. Tattikota SG, Rathjen T, McAnulty SJ, Wessels HH, Akerman I, van de Bunt M, Hausser J, Esguerra JL, Musahl A, Pandey AK, You X, Chen W, Herrera PL, Johnson PR, O'Carroll D, Eliasson L, Zavolan M, Gloyn AL, Ferrer J, Shalom-Feuerstein R, Aberdam D, Poy MN. [Argonaute2 mediates compensatory expansion of the pancreatic β cell.](http://www.ncbi.nlm.nih.gov/pubmed/24361012) **Cell Metabolism.**  **2014** Jan 7;19(1):122-34.

**Articles that were submitted to publication and are now under reviewing or revision:**

1. Corneal committed cells restore the stem cell pool and tissue boundary following injury. Waseem Nasser, Aya Amitai-Lange, Despina Soteriou, Rana Hanna, Beatrice Tiosano, Yaron Fuchs, Ruby Shalom-Feuerstein1. *Submitted*.
2. Sox2 controls stemness, proliferation and epigenetic landscape of corneal epithelial cells. Swarnabh Bhattacharya, Laura Serror, Eshkar Nir, Dhiraj Dalbir, Beatrice Tiosano, Peleg Hasson, Lia Panman, Ruby Shalom-Feuerstein. *Under review*.
3. microRNA-184 induces a commitment switch to epidermal differentiation. Sara Nagosa, Friederike Leesch1, Daria Putin, Swarnabh Bhattacharya, Anna Altshuler, Laura Serror, Aya Amitai-Lange, Waseem Nasser, Edith Aberdam, Matthieu Rouleau, Sudhir G. Tattikota, Matthew N. Poy, Daniel Aberdam, Ruby Shalom-Feuerstein. *Revised*.
4. RAS regulates the transition from naïve to primed pluripotent stem cells. Anna Altshuler, Mila Verbuk, Swarnabh Bhattacharya, Roni Haklai, Yoel Kloog, Eran Meshorer, Ruby Shalom-Feuerstein. *Under review*.

**Section B (Confidential (5) or public: confidential information to be marked clearly)**

**TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, UTILITY MODELS, ETC.**

Add or modify applications

N/A

(5) Not to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

Please complete the table hereafter:

N/A

**TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND**

N/A

**ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND**

Add or modify exploitable foregrounds

N/A

(6) In the table, for each row, please provide a text to explain the exploitable foreground, in particular: - Its purpose - How the foreground might be exploited, when and by whom - IPR exploitable measures taken or intended - Further research necessary, if any - Potential/expected impact (quantify where possible)

**3. SCIENTIST IN CHARGE QUESTIONNAIRE**

**RESEARCH TRAINING ASSESSMENT:**

What is the size of the hosting research group?

**How many researchers have you supervised, within the past 10 years? Of which funded by:**

EC/Marie Curie actions:\* 4

EC Other Funding:\* NA

University fellowships:\*4

National public bodies:\*5

Industry:\*0

Other:\*0

Other, please specify:

How many researchers have you supervised within this project?\*13

Corresponding to how many person months?\*290

**Number of publications resulting directly from the research project:**

Recruited researcher(s) and yourself\* 5 published+4 were submitted, are in process

Recruited researcher(s) alone\*

Recruited researcher(s) with authors other than yourself\*

**Participation of the recruited researcher(s) at conferences (number):**

Passive\*2

Active\*23

How do you rate the overall success of the research training?

Very good

General assessment:

**RESEARCHERS ASSESSMENT:**

**Rate the overall level of the recruited researcher(s) integration in the research team and the host organization with regards to:**

Participation in meetings/seminars: Very good

Discussions of results and project-related topics: Very good

Co-operation with other team members: Very good

Co-operation with other researchers of the host institution: Very good

**Rate the overall performance of the recruited researcher(s) with regard to:**

Originality of fellow(s) approach towards research (initiative/independent thinking):

Good

Capacity to develop new skills and to benefit from training: Very good

Productivity (research results/publications/international conference attendance): Very good

Communication skills: Very good

Group leader skills (collaboration with other groups/project management): Good

Training and/or teaching skills: Very good

Please comment:

**RESEARCH TRAINING OUTCOMES:**

**Has this project provided additional links with other research groups or institutions?**

Yes

If yes, indicate the number of contacts in each case.

If Other, please specify:

**Rate the importance of the following outcomes of the research training:**

Results of the research: Very good

Number of publications: Very good

Development of research: Very good

Establishment of international collaborations: Very good

Transfer of knowledge/technology: Very good

Training of students/researchers: Very good

Further academic qualifications (PhD, habilitation etc.) for fellows: Very good

Please comment:

**YOUR OPINION ABOUT THE MARIE CURIE ACTIONS**:

Do you have any other comments or suggestions of how to improve the concerned Marie Curie actions?

**Did you have previous knowledge of the Marie Curie actions?**

Yes

If yes, what sort of image do you think that the Marie Curie actions have among the scientific community in your research area? Very good