



UNIVERSITÀ DEGLI STUDI DI PALERMO

Dipartimento Biomedico di Medicina Interna e Specialistica  
Sezione di Endocrinologia, Diabetologia e Metabolismo

Dottorato di ricerca in “Genomica e Proteomica nella ricerca  
oncologica ed endocrino metabolica” XXIV ciclo

***Isolation, genomic and proteomic  
characterization of fibroblastic and epithelial  
limbal stem cells and evaluation of their  
multilineage differentiation capability.***

Settore Scientifico disciplinare  
MED. 13/ENDOCRINOLOGIA

Tesi di Dottorato  
Dott.ssa Laura Tomasello

Tutor  
Prof.ssa Carla Giordano  
Coordinatore  
Prof. Salvatore Feo

## ABSTRACT

**BACKGROUND:** The limbus of the eye, located at the junction of the cornea and conjunctiva of the ocular surface, represents a unique stem cell niche in human body. A critical advantage of limbal cells is that they are easily accessible with a well established and minimally invasive procedure. Several groups have reported the gene expression profile of limbal and corneal epithelial cells that has significantly contributed to the understanding of several cellular pathways and intrinsic factors that underpin the phenotypic difference between the two cell types [1-3]. However the gene expression profile of the human limbal epithelial and limbal stromal cultured cells obtained from the native limbal tissue has not been addressed until now. The lack of specific molecular markers for the identification of the multipotent limbal subpopulation limited the investigation of their differentiation capability. SSEA4 (stage specific embryonic antigen) is recognized marker of pluripotent stem cells, such as embryonic stem cells, and not of multipotent stem cells. Actually, the researcher are agreed that SSEA4 expression is lost from stem cells as they start to differentiate. It is generally agreed that the LESC are characterized by special location in the limbus, clonality, cytokeratin profile, transformation-related protein 63 (p63) delta isomers, and ATP-binding cassette sub-family G member 2 (ABCG2) expression [4-5]. Fibroblast (f)-LSCs represent a multipotent stem cell population characterized as CD105+, CD73+, CD90+, CD34+ and CD45- as well as by their self-renewal and multi-lineage differentiation capability [6-7].

AIM: To identify the better protocol of isolation and expansion for LESC and f-LSCs to obtain two purified and distinct subpopulations for molecular and proteomic characterization to evaluate their multilineage differentiation capability.

MATERIAL AND METHODS: Cytofluorimetric analysis; sphere formation assay; qRT PCR and proteomic assays; multilineage differentiation assays were performed.

RESULTS: Both f-LSC and LESC populations expressed SSEA4 (principal staminal marker)  $98.63 \pm 3.5\%$ ; vs  $78.32 \pm 2.8\%$ , respectively (at 2PD) and ABCG2 (limbal specific marker).

The f-LSC genomic expression profile in comparison to LESC didn't show significant differences and both cell populations kept a stem genomic expression profiles since 24 PD. f-LSC kept an high SSEA4 positive expression ( $98.63 \pm 3.5\%$ ;  $90.36 \pm 1.4\%$ ; 2PD-14PD) whereas LESC showed a CK15<sup>+</sup>/ΔNp63<sup>+</sup> pattern and a reduced expression of SSEA4 ( $78.32 \pm 2.8\%$  vs  $13.90 \pm 4.6\%$ ; 2PD-14PD).

f-LSC, but not LESC, were differentiated into pancreatic β-cell phenotype (insulin-producing beta-like cells). Furthermore, both f-LSC and LESC achieved terminal osteoblastic and adipose differentiation. By contrast, the specific for committed LESC towards definitive epithelial phenotype cytokeratin profile, reduced LESC multilineage differentiation skill.

CONCLUSIONS: The identification of genetic profiles and novel molecular markers characteristic of specific cellular phenotypes is likely to assist in better defining the stages of

progression from unspecialized cells to cell types of interest. Yet, translation of these promising basic science discoveries into successful cell replacement therapy in human subjects is likely to require sophisticated methods for assessing genetic and phenotypic stability.

#### REFERENCES:

1. Kheirkhah, A.; Raju, V.K.; Tseng, S.C. Minimal conjunctival limbal autograft for total limbal stem cell deficiency. *Cornea* 27(6):730-733; 2008. 22.
2. Lee, C.S.; Friedman, J.R.; Fulmer, J.T.; Kaestner, K.H. The initiation of liver development is dependent on Foxa transcription factors. *Nature* 435(7044):944- 947; 2005.20-22].
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-7.
4. De Paiva, C.S.; Chen, Z.; Corrales, R.M.; Pflugfelder, S.C.; Li, D.Q. ABCG2 2 transporter identifies a population of clonogenic human limbal epithelial cells. *Stem Cells* 23(1):63-73; 2005.4 10.
5. Di Iorio, E.; Barbaro, V.; Ruzza, A.; Ponzin, D.; Pellegrini, G.; De Luca, M. .5 Isoforms of DeltaNp63 and the migration of ocular limbal cells in human6 corneal regeneration. *Proc. Natl. Acad. Sci. Usa.* 102(27):9523-

9528; 2005.).

6. Chen, Z.; Teo, A.; Pedersen, R.A.; Vallier, L. SIP1 Mediates Cell-Fate Decisions between Neuroectoderm and Mesendoderm in Human Pluripotent Stem Cells. *Cell Stem Cell* 6(1):59-70; 2010.
7. Criscimanna A, Zito G, Taddeo A, Richiusa P, Pitrone M, Morreale D, Lodato G, Pizzolanti G, Citarrella R, Galluzzo A, Giordano C. In vitro generation of pancreatic endocrine cells from human adult fibroblast-like limbal stem cells. *Cell Transplant*. 2012;21(1):73-90.