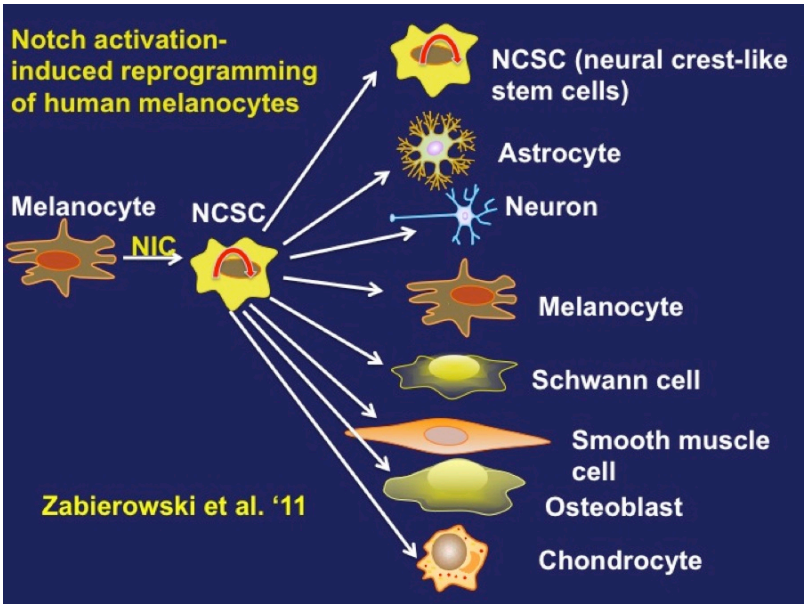
Stem Cells

Induced Stem Cells

Mouse and human somatic cells can either be reprogrammed to a pluripotent state or converted to another lineage with a combination of transcription factors suggesting that lineage commitment is a reversible process. Here we show that only one factor, the active intracellular form of Notch1, is sufficient to convert mature pigmented epidermal-derived melanocytes into functional multipotent neural crest stem-like cells. These induced neural crest stem cells (iNCSCs) proliferate as spheres under stem cell media conditions, re-express neural crest-related genes and differentiate into multiple neural crest derived mesenchymal and neuronal lineages. Moreover, iNCSCs are highly migratory and functional *in vivo*. These results demonstrate that mature melanocytes can be reprogrammed toward their primitive neural crest cell precursors through the activation of a single stem cell-related pathway.

Zabierowski, S.E., Baubet, V., Himes, B, Li, L., Fukunaga-Kalabis, M., Patel, S., McDaid, R., Guerra, M., Gimotty, P., Dahamne, N., and Herlyn, M.: Direct reprogramming of melanocytes to neural crest stem-like cells by one defined factor. Stem Cells 11: 1752-62, 2011. PMID21948558.



Melanoma Stem Cells

The utility of different models to identify cancer stem cells continues to be a subject of intense debate.

Our long-term goal is to define the microenvironment within malignant lesions, i.e. matrix and stromal cells, that is responsible for self-renewal of malignant cells and that likely also controls proliferation and invasion. Our initial studies were challenging because the melanoma stem cell population, as defined by us through CD20, comprises only 0.1 to 1% of the total population.

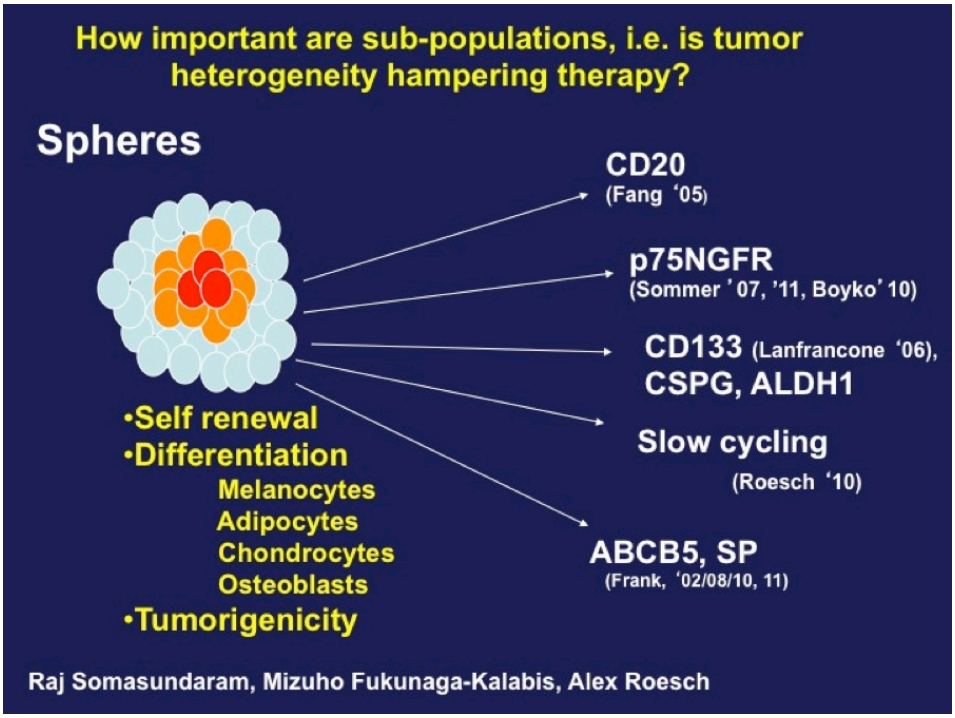
Fang, D., Nguyen, T.K., Leishear, K., Finko, R., Kulp, A.N., Hotz, S., Van Belle, P.A., Xu, X., Elder, D.E., Herlyn, M.: A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 65:9328-9337, 2005. PMID16230395

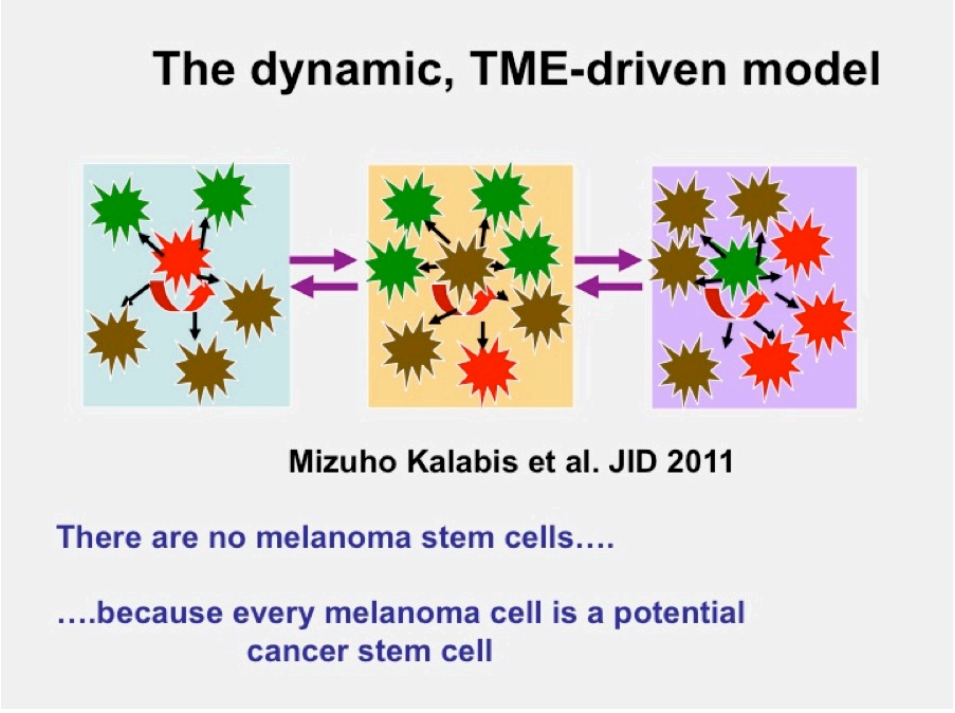
We have then characterized intra-tumoral heterogeneity of melanoma. Within a developing or already established tumor microenvironment, we propose that continuous tumor maintenance is assured by specific subpopulations whose phenotype is not static but instead is dynamically regulated. These small and temporarily distinct subpopulations likely play critical roles in tumor progression. They are important therapeutic targets but only in the context of combination therapies that also eliminate the bulk of the tumor. Using the H3K4 demethylase JARID1B (KDM5B/PLU-1/RBP2-H1) as a biomarker, we have characterized a small subpopulation of slow-cycling melanoma cells that cycle with doubling times of >4 weeks within the rapidly proliferating main population. Isolated JARID1B-positive melanoma cells give rise to a highly proliferative progeny. Knock-down of JARID1B leads to an initial acceleration of tumor growth followed by exhaustion which suggests that the JARID1B-positive subpopulation is essential for continuous tumor growth. Expression of JARID1B is dynamically regulated and does not follow a hierarchical cancer stem cell model because JARID1B-negative cells can become positive and even single melanoma cells irrespective of selection are tumorigenic. These results suggest a new understanding of melanoma heterogeneity with tumor maintenance as a dynamic process mediated by a temporarily distinct subpopulation.

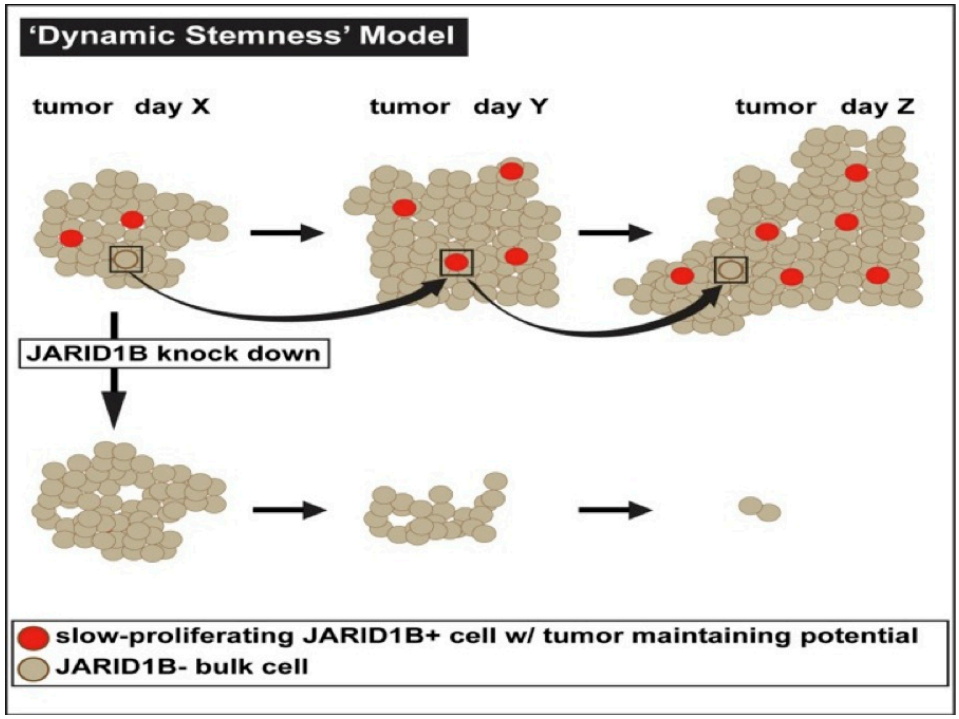
Roesch, A., Fukunaga-Kalabis, M., Schmidt, E.C., Zabierowski, S.E., Brafford, P.A., Vultur, A., Basu, D., Gimotty, P., Vogt, T., Herlyn, M.: A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell 141: 583-594, 2010. PMID204782552 (PMC2882693).

Fukunaga-Kalabis, M., Roesch, A., Herlyn, M.: From cancer stem cells to tumor maintainenance cells. J Invest. Dermatol. 131: 1600-1604, 2011. PMID 21654838

Characterization of sub-populations with stem cell-like properties



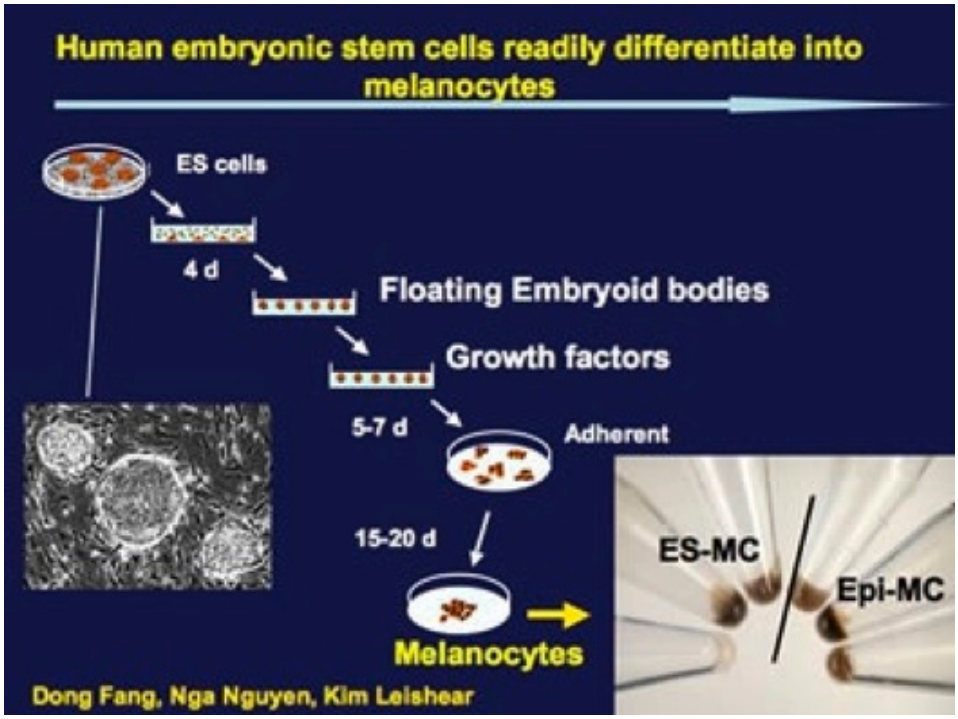


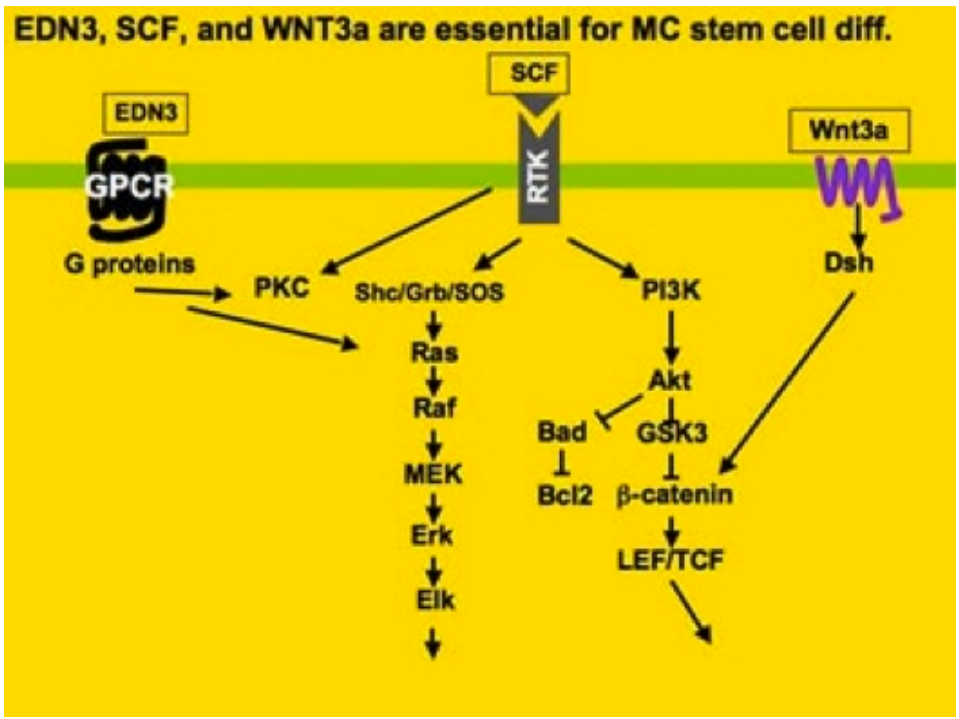


ES Cells

Human embryonic stem cells readily differentiate into human melanocytes and are thus an ideal model to investigate the stages of differentiation. The most important growth factors for differentiation are Wnt 3a, SCF and EDN3. Although we can readily induce their differentiation, we do not yet know exactly the mechanisms for differentiation nor have we yet defined the precursor stages.

Fang, D., Leishear, K., Nguyen, T.K., Finko, R., Cai, K., Fukunaga, M., Li, L., Brafford, P.A., Kulp, A.N., Xu, X., Smalley, K.S., Herlyn, M.: Defining the conditions for the generation of melanocytes from human embryonic stem cells. Stem Cells 24:166 8-1677, 2006.
Zabierowski, S.E., Herlyn, M.: Embryonic stem cells as a model to study melanocyte development. Methods Mol Biol. 584: 301-316, 2010. PMID 19907984


Human embryonic stem cells readily differentiate into melanocytes
ES cells
4 d
Floating Embryoid bodies
Growth factors
5-7 d
Adherent
15-20 d
Melanocytes
ES-MC
Epi-MC
Dong Fang, Nga Nguyen, Kim Leishear

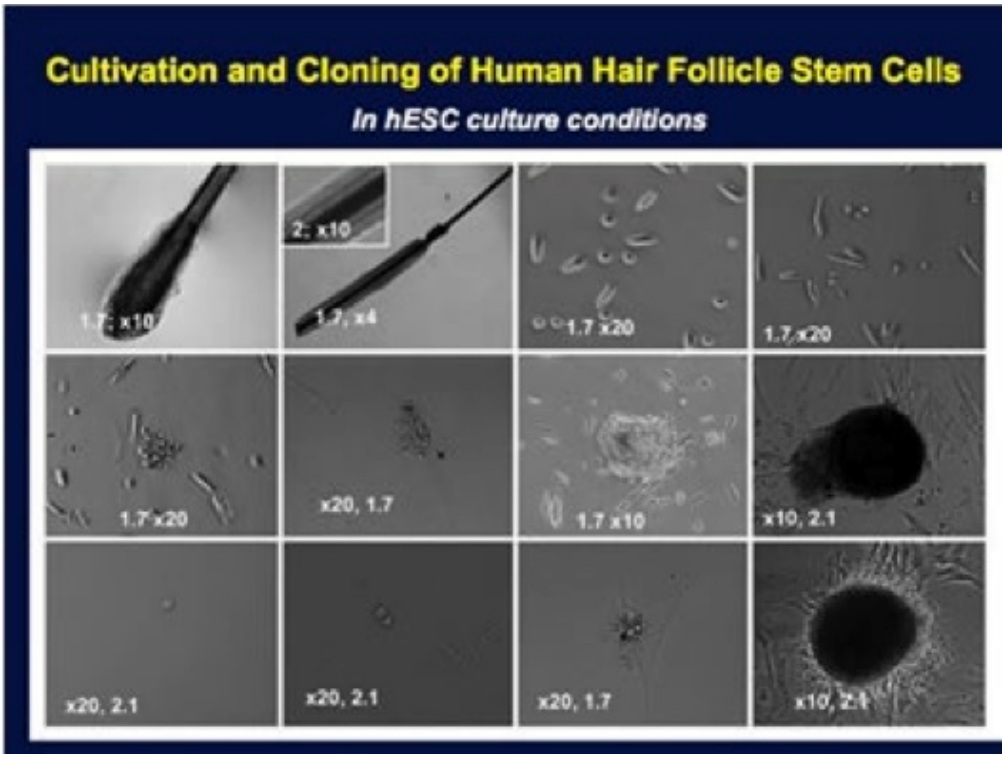

EDN3, SCF, and WNT3a are essential for MC stem cell diff.
SCF
EDN3
Wnt3a
GPCR
RTK
G proteins
PKC
Shc/Grb/SOS
PI3K
Dsh
Ras
Raf
MEK
Erk
Elk
Akt
Bad
GSK3
Bcl2
β-catenin
LEF/TCF

Hair Stem Cells

The physiological life cycle of melanocytes in human skin is likely very different from mouse skin since murine melanocytes are almost exclusively located in the hair follicle and dermis and not in the epidermis. In contrast to keratinocyte stem cells, melanocyte stem cells in human skin were only very recently identified in the hair follicle. Our laboratory has isolated and characterized a multi-potent progenitor cell from the human hair follicle and maintained it in 'hair spheres' that can differentiate not only into melanocytes but also into neuronal and smooth muscle cells. We learned to culture these cells as hair spheres using media conditions developed for human embryonic stem cells, which we can also differentiate into melanocytes. Multi-potent progenitor cells, grown as 'embryoid bodies' from embryonic stem cells or hair spheres from hair follicles, will provide the main resource for our studies in identifying the microenvironmental cues for stem cell differentiation in skin.

The key for our understanding of self-renewal and differentiation is composition and physical stiffness of the matrix. Nothing is known about the stem cell niche for human melanocytes outside of the human hair follicle, but on several locations of the human body the skin is normally pigmented although there are no hair follicles. In preliminary studies we have isolated spheres from both epidermis and dermis of foreskin that contain cells capable of differentiating into melanocytes and appear to have multi-potent characteristics because they can also differentiate into neuronal cells, smooth muscle cells and adipocytes.

Yu, H., Fang, D., Kumar, S.M., Li, L., Nguyen, T. K., Acs, G., Herlyn, M., Xu, X.: Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol 168:1879-1988, 2006. PMID16723703



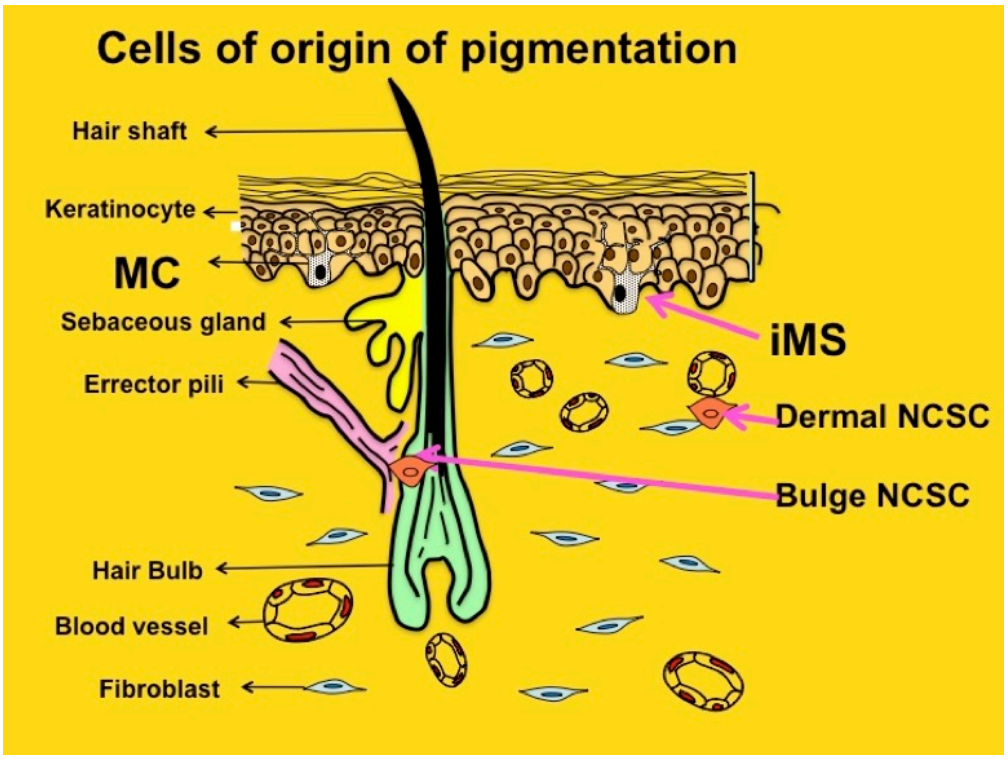
Multi-Potent Dermal Cells

Multipotent dermal stem cells, isolated from human foreskins, lacking hair follicles, are able to home to the epidermis to differentiate into melanocytes. These dermal stem cells, grown as three-dimensional spheres, displayed capacity for self-renewal and expressed NGFRp75, nestin and OCT4, but not melanocyte markers. In addition, cells derived from single cell clones were able to differentiate into multiple lineages including melanocytes. In a three-dimensional skin equivalent model, sphere-forming cells differentiated into HMB45-positive melanocytes that migrated from the dermis to the epidermis and aligned singly among the basal layer keratinocytes in a similar fashion to pigmented melanocytes isolated from the epidermis. The dermal stem cells were negative for E-cadherin and N-cadherin, while they acquired E-cadherin expression and lost NGFRp75 expression upon contact with epidermal keratinocytes.

Li, L., Fulunaga-Kalabis, M., Yu, H., Xu, X., Kong, J., Lee, J.T. Herlyn, M.: Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci 123: 853-860, 2010. PMID20159965 (PMC2831759)

Zabierowski, S.E., Fukunaga-Kalabis, M., Li, L., Herlyn, M.: Dermis-derived stem cells: A source of epidermal melanocytes and melanoma? Pigment Cell Melanoma Res 24: 422-429, 2011. PMID 21410654
Li, L., Fukunaga-Kalabis, M., Herlyn, M.: Isolation and cultivation of dermal stem cells that differentiate into functional melanocytes. In: Human cell culture protocols. Methods in Molecular Biology 806:15-29, 2012. PMID22057442

Cells that can transform to melanoma



NSCS = Neural crest stem cells
MC = Melanocytes
IMS = Induced multi-potent stem cells