Research Area A – Cancer Cell Dissemination

Project Package A1: Cancer Stem Cells (Projects 1-4)

Project 2: **Characterization of human melanoma cells on the basis of markers of pluripotent stem cells**

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Short Summary

Embryonic stem cells (ES cells) are similar to melanoma cells in many aspects. ES cells are immortal and proliferate rapidly. They also form tumors (teratomas) when transplanted into immune-deficient mice. Similar to ES cells melanoma cells show also a plasticity. By the ectopic overexpression of different sets of transcription factors or microRNAs somatic cells can be converted into ES-like cells.

Our studies indicate that melanoma comprise different subpopulations which express marker of pluripotent stem cells (e.g. Nanog, Sox2). The projects objective will be to identify such subpopulations within murine and human primary melanoma cells as well as in melanoma cell lines. Respective subpopulations will be analyzed at the genomic, epigenomic, and proteomic level. Functional abilities for maintaining the tumor cell growth will be tested. Moreover, the accessibility of different subpopulations towards cellular reprogramming, the reprogramming kinetics and factor requirements will be investigated. This project should help to understand the maintenance and formation of melanomas. The examined markers might serve as therapeutic targets in future.

3 State of the Art

3.1 State of knowledge in the field

Embryonic stem cells (ES cells) are similar to tumor cells in many aspects. ES cells are immortal and proliferate rapidly. They also form tumors (teratomas) when transplanted into immune-deficient mice.

3.2 Preliminary work by the participants

We have shown that different cell types including mouse and human melanocytes or melanoma cells can be reprogrammed into pluripotent stem cells by the ectopic expression of transcription factors such as Oct4, Klf4, Sox2 and c-Myc. These pluripotent stem cells have all the features of ES cells including immortal growth, the expression of pluripotency markers (e.g. Sox2 and Nanog) and the potential of forming teratomas (Stadtfeld et al., 2008; Eminli et al., 2008; Utikal et al., 2009a). The conversion efficiencies of melanocytes into pluripotent stem cells can be increased dramatically by downregulating p53 or p16/p19 further underscoring similarities of this mechanism with tumorigenesis (Utikal et al., 2009b). Our preliminary studies show that subpopulations of human melanoma cells reveal an endogenous expression of pluripotency markers such as Nanog or Sox2. However, these cell populations are not yet well characterized and their functional abilities for maintaining the tumor cell growth are not yet known.

4 Project Plan

4.1 Specific Aims

Main hypothesis: Markers of pluripotent stem cells play a main role in development and maintenance of human malignant melanoma

Aim 1: Identification of cell populations which express markers of pluripotent stem cells (e.g. Sox2, Nanog) in murine and human primary melanoma cells and melanoma cell lines.

Aim 2: In-depth analysis and comparison of expression profile and epigenetic status as well as functional analysis of different subpopulations.

4.2 Experimental program

In order to identify subpopulations of melanoma cells expressing common markers of pluripotent stem cells such as Sox2 or Nanog lentiviral reporter constructs will be generated. Reporter constructs will be designed such that the promoter of a particular pluripotency marker will control the co-expression of a fluorescing (e.g. GFP) and an antibiotic selection marker. Lentiviral particles carrying the reporter constructs will be produced and purified.

Primary melanoma cells directly isolated from patients, from our established transgenic RET melanoma mouse model as well as cells from melanoma cell lines (e.g. C32, HT144) will be infected with lentiviral vectors that carry the reporter constructs. Cells expressing the respective genes and accordingly also expressing the fluorescent marker will be visualized by means of fluorescent microscopy. Quantification and separation of fluorescently labelled Sox2- and Nanog-expressing melanoma cells will be done by fluorescence activated cell sorting. This will also enable to monitor if cells from a certain subpopulation have a stable phenotype or might perhaps convert to cells from a different subpopulation.

The sorted populations will be compared by analysing the expression of additional stem cell markers, global gene expression and DNA methylation in detail. Immunofluorescent labeling will be performed to examine the expression of markers (e.g. Sox2, Nanog, SSEA-3/4, Lin28) and melanoma-specific markers (e.g. S100, MART-1, HMB-45, MITF), respectively. For the evaluation of differential gene expression DNA microarrays and RT-PCR will be performed. Moreover, the promoter methylation status of stem cell- and melanoma-associated genes as an indicator for transcriptional activity will be checked by bisulfite sequencing.

To correlate the afore mentioned parameters with functional properties, the tumorigenic potential of subpopulations will be investigated. For this purpose, the cells will be injected subcutaneously into immune-deficient mice (in the case of human cells) or in the syngenic RET transgenic melanoma mouse model (mouse melanoma cells) and tumor growth will be quantified. By knocking down the expression of melanoma- and pluripotency-associated genes with shRNA, the role of these genes for tumor development and growth will be studied. The impact of different subpopulations on tumor development will be analyzed by specifically depleting those cells. This will be achieved by infecting melanoma cells with a lentiviral construct that contains a promoter (e.g. Nanog or Sox2 promoter) which controls the expression of the enzyme thymidine kinase. Application of ganciclovir will selectively deplete all cells which express thymidine kinase. Tumor growth in the absence and presence of different subpopulations will be compared and marker switching abilities of tumor cells will be investigated. Another functional aspect we will focus on is the amenability of melanoma subpopulations to the process of reprogramming by transcription factors such as ectopic Oct4, Klf4 and c-Myc. Reprogramming kinetics and factor requirements will be determined.

4.3 Collaborations with other Projects in the RTG

For the analysis of melanoma subpopulations at the genomic, epigenetic, and proteomic level, we will closely collaborate with projects 1 and 4. Functional abilities for initiating and maintaining tumor cell growth will be tested in cooperation with project 10. The ret transgenic mouse model of malignant melanoma will be provided by project 13.

5 References

1. Eminli S*, **Utikal J***, Arnold K, Jaenisch R, Hochedlinger K. 2008. Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. *Stem Cells* 26:2467-74. * authors contributed equally
2. Stadtfeld M, Nagaya M, **Utikal J**, Weir G, Hochedlinger K. 2008. Induced pluripotent stem cells generated without viral integration. *Science* 322:945-9.
3. **Utikal J**, Maherali N, Kulalert W, Hochedlinger K. 2009. Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. *J Cell Science* 122:3502-10.

4. **Utikal J**, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG, Hochedlinger K. 2009. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 460:1145-8.