

EPITHELIAL-MESENCHYMAL TRANSITION IN MELANOMAGENESIS

Helmut Schaidler

Cancer Biology Unit, Department of Dermatology,
Center of Molecular Medicine, Medical University of Graz

Email: helmut.schaidler@klinikum-graz.at

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ABSTRACT *Epithelial-mesenchymal transition (EMT) has been described as a fundamental process for solid cancers originating from epithelial cells during early tumorigenesis with implications for cancer progression and metastases. Whether the concept of EMT is also valid for melanocytic cells, which are derived from neural-crest cells, is unknown. One important step is the E- to N-cadherin switch, which is presumably accomplished through epithelial-mesenchymal transcriptional regulators (EMTRs). We hypothesize that EMTRs are principally involved in every step of early melanomagenesis, initiation of melanoma development and progression.*

INTRODUCTION AND PREVIOUS WORK

Repression of *CDH1*, the gene encoding E-cadherin, is considered as a crucial and initial step in dissociating and migration of tumor cells from the primary site of tumor development (1). This is accomplished through transcription factors, such as the Snail and basic helix-loop-helix (bHLH) families, that strongly repress *CDH1* and other genes that encode integrins, cytokeratins and occludin, to name a few. These are cited as epithelial-mesenchymal transcriptional regulators (EMTRs), representatives are Snail and Slug of the Snail family and Twist of the bHLH family (2). We have previously shown that the growth factor HGF downregulates E-cadherin and Desmoglein 1 in melanocytic cells (3). However, mechanisms of the downregulation are unknown, thus we questioned if EMTRs may be targets of HGF modulation. To this end, we determined whether the expression of EMTRs changes after exposure to rhHGF, depending on the respective endogenous protein levels. In melanoma cells, isolated from metastases (WM9), Slug and Snail are profoundly downregulated (Fig. 1) after exposure to rhHGF, which was confirmed by adenoviral transduction (not shown). Snail and Slug expression was completely abrogated after 48hrs, whereas Twist increased in the nucleus after exposure to rhHGF, with a steady decline in the cytoplasm. These results point to a differential modulation of EMTRs through HGF, which finally may impact the expression status of E- and N-cadherin. Currently, binding properties of EMTRs to the promoter regions of E- and N-cadherin are determined by gel-shift assays.

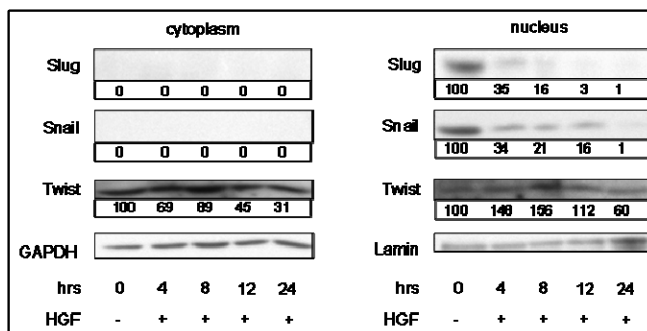


Fig. 1 Western blot analyses of Snail, Slug and Twist expression in a metastatic melanoma cell line (WM9). Time-dependent changes in expression after exposure to rhHGF (50ng/ml) up to 24 hrs. Equal aliquots (10µg) of nuclear and cytoplasmic extracts were loaded on a 10% SDS-polyacrylamide gel. GAPDH and Lamin served as loading controls for cytoplasmic and nuclear extracts respectively. Numbers indicate changes in percent of un-exposed control.

PhD THESIS Several EMTRs, like Snail, Slug and Twist have been identified as transcriptional repressors of E-cadherin. The mechanisms responsible for activated EMTR expression and involvement of co-activators or -repressors are unknown. Likewise, influence of EMTRs on phenotypic changes in melanocytic cells and the impact on melanoma initiation and progression are to be determined.

Aim1. To identify co-repressors and –activators of EMTRs in melanocytic cells

Aim2. To define transforming capacities of EMTRs towards a mesenchymal phenotype

Aim3. To determine if EMTRs are driving melanoma initiation and progression in vivo

Outline of the approach. Melanocytic cells (primary human melanocytes, melanoma cells from different stages) are used throughout *Aims 1-3*.

Aim1. We continue to characterize nuclear protein complexes, which constitute the shifts of un-stimulated and stimulated melanocytic cells in gel-shift assays. Elutes of in-gel digestions are subjected to mass spectrometry for peptide mass fingerprinting.

Aim2. We will test the hypothesis that EMTRs significantly contribute to the transition of an epithelial to a mesenchymal phenotype in melanocytic cells. Through over-expression or down-regulation of EMTRs via lentiviral and shRNA vectors we assess for functionality by assays for constriction of collagen, synthesis of extracellular matrix proteins, 3-D network formation assays and *in vitro* skin reconstructs. We expect that EMTRs are inducing a phenotype in melanocytic cells, which enables them to cope with environmental conditions found in the dermis of human skin.

Aim3. We will test the hypothesis that EMTRs are pivotal for early steps and progression in melanomagenesis *in vivo*. To examine, whether single expression of EMTRs is critical for melanoma induction, we will generate transgenic mice expressing Slug or Twist under the control of regulatory elements from the Microphthalmia-associated transcriptional factor (*Mitf*) -M gene. We expect that EMTRs drive a genetic program with profound changes leading to an initiation process.

REFERENCES

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