MOLECULAR MECHANISMS OF EMBRYONIC STEM CELL PLURIPOTENCY: TRANSCRIPTION, TELOMERE MAINTENANCE AND PROLIFERATION

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av

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Avhandlingen baseras på följande delarbeten:

I. **Vizlin-Hodzic, D**., Johansson, H., Ryme, J., Simonsson, T., Simonsson, S. SAF-A has a role in transcriptional regulation of *Oct4* in ES cells through promoter binding. Cellular reprogramming, *In press* (2010)

II. **Vizlin-Hodzic, D.***, Ryme, J.*, Runnberg, R., Simonsson, S., Simonsson, T. SAF-A together with Brg1 is required for RNA polymerase II mediated transcription. *Submitted manuscript*
*contributed equally to this work

III. **Vizlin-Hodzic, D.**, Johansson, H., Jemt, E., Horvath, G., Simonsson, T., Simonsson, S. Oct4 as a Prognostic Biomarker of Ovarian Cancer. *Manuscript*

IV. **Vizlin-Hodzic, D**., Ryme, J., Simonsson, S., Simonsson, T. Developmental studies of *Xenopus* shelterin complexes: the message to reset telomere length is already present in the egg.
FASEB J, 23; 2587-2594. (2009)

V. Johansson, H., **Vizlin-Hodzic, D.**, Simonsson, T., Simonsson, S. Translationally controlled tumor protein interacts with nucleophosmin during mitosis in ES cells. Cell Cycle, 9; 2160-2169. (2010)

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Abstract

Somatic cell nuclear transfer and generation of induced pluripotent stem cells provide potential routes towards generation of patient specific embryonic stem (ES) cells. These procedures require induction of *Oct4* gene expression, high telomerase activity and specific cell proliferation, characteristics shared with cancer stem cells. The aim of this thesis is to gain further understanding of the molecular mechanisms that control these events.

In an attempt to identify factors involved in transcriptional regulation of the *Oct4*, the binding of SAF-A with the *Oct4* proximal promoter region in a LIF signalling dependent manner was established and subsequently demonstrated to be of functional importance for *Oct4* transcription. Further investigations revealed SAF-A in complex with proven affecters of *Oct4* transcription, Oct4 itself and Sox2, as well as with RNA polymerase II indicating that SAF-A could serve to bring together factors required for *Oct4* transcription and load them on the promoter. Moreover, SAF-A was found in a complex with the SWI/SNF-Brg1 chromatin remodelling protein in ES and differentiation induced cells. Functional assays revealed that dual depletion of SAF-A and Brg1 abolishes global transcription by RNA polymerase II indicating a fundamental role for the complex in RNA polymerase II mediated transcription.

The *Oct4* expression, as well as its transcriptional regulation were investigated in the biopsy samples from ovarian cancer patients. This investigation revealed reactivation of the *Oct4* expression independently of epigenetic regulation in biopsy samples from ovarian cancer patients. Further, these patients survived no more than 3.5 years from the diagnosis suggesting that Oct4 could be used as a prognostic factor of ovarian cancer mortality.

Telomere extension by telomerase is mediated by the shelterin complexes. The identification and biochemical characterization of the telomere shelterin complexes in *Xenopus* revealed conservation of their main functions in relation to human orthologs. Moreover, the temporal regulation of shelterin composition and subcomplex appearance was demonstrated during *Xenopus* embryonic development.

In screening for Tpt1 interacting factors in ES cells, Npm1 was found. The interaction occurred in a cell cycle dependent manner and subsequent functional assays proved its involvement in cell proliferation.

In conclusion, new insights regarding *Oct4* transcriptional regulation, telomere maintenance and ES cell proliferation are presented in this thesis.

Key words: embryonic stem cells, Oct4, SAF-A, Brg1, Tpt1, Npm1, transcriptional regulation, cell proliferation, shelterin

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