Identification of stem cell factors

Novel protein-protein interactions and their functions

Akademisk avhandling

som för avläggande av medicine doktorsexamen
vid Sahlgrenska Akademin vid Göteborgs universitet kommer att offentligen försvaras
i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg,
fredagen den 1 oktober 2010, kl. 09.00

av

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Avhandlingen baseras på följande delarbeten.

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

II. **Johansson, H.,** Svensson, F., Simonsson, T., Simonsson, S.
Phosphorylated nucleolin interacts with translationally controlled tumor protein during mitosis and with Oct4 during interphase in ES cells.
Submitted manuscript, under revision

III. Vizlin-Hodzic, D., **Johansson, H.,** Simonsson, T., Simonsson, S.
SAF-A has a role in transcriptional regulation of *Oct4* in ES cells through promoter binding.
Submitted manuscript

IV. **Johansson, H.,** and Simonsson, S.
Nucleophosmin is in complex with Oct4, Sox2 and Nanog in ES cells.
Manuscript

Göteborg 2010

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Novel protein-protein interactions and their functions

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Abstract

Embryonic stem (ES) cells provide an unlimited source of cells potentially useful for regenerative medicine, however, prior to clinical implementation, additional basic research is needed. This thesis is focused upon different molecular aspects regarding ES cells, primarily by finding novel stem cell protein-protein interactions and their functions. As functions of a specific protein may be dependent on its interacting partner, identification of such protein-protein interactions is important. Using several different methods, for example *in situ* proximity ligation assay and co-immunoprecipitation, numerous novel protein-protein interactions occurring in ES cells were found. The same proteins were shown to be involved in several different protein complexes, some of them likely to be part of bigger complexes. Tpt1 and Npm1 were two such proteins found in several different interactions. Tpt1/Npm1 interacted with a prominent peak during mitosis and were proven to be involved in cell proliferation. Individual depletion of *Tpt1* and *Npm1* resulted in increased levels of markers of the neural and mesodermal lineages, respectively. Further, Npm1 also associated with all three core transcription factors, namely Oct4, Sox2 and Nanog, signifying the importance of Npm1 in ES cells. The Npm1/Sox2 interaction was shown to remain while cells were induced to differentiate into neural lineage, while decreasing in the other differentiation pathways, indicating of an additional role of this protein complex during differentiation to ectoderm. Phosphorylated Ncl was found to interact individually with Tpt1 and Oct4 in a cell cycle dependent manner, speculatively involved in cell proliferation and transcription. In screening for factors binding to *Oct4* proximal promoter, SAF-A was found and subsequently shown to be involved in the transcriptional regulation of *Oct4*. The binding occurred preferentially to unmethylated *Oct4* promoter and was reduced when ES cells were induced to differentiate. SAF-A was also found to interact with RNA pol II as well as STAT3, Oct4 and Sox2. In conclusion: twelve novel protein-protein interactions, involved in cell proliferation, differentiation and transcriptional regulation, are presented in this thesis.

Key words: embryonic stem cells, Tpt1, Npm1, Ncl, Oct4, Sox2, Nanog, SAF-A, cell proliferation, transcriptional regulation

ISBN: 978-91-628-8135-1