Karolinska Institutet

Institutionen för Cell- och Molekylärbiologi

Linker Histone H1 and Androgen Receptor: Two Different Players in the Chromatin Orchestra

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Institutionen för Cell och Molekylärbiologi (CMB) auditorium, Berzelius väg 21

Torsdagen den 14 juni, 2012, kl 9.00

av

Christine Öberg

Huvudhandledare:
Docent Sergey Belikov
Karolinska Institutet
Institutionen för Cell-och Molekylärbiologi

Bihandledare:
Professor Örjan Wrange
Karolinska Institutet
Institutionen för Cell-och Molekylärbiologi

Fakultetsopponent:
Professor Ingemar Rundquist
Linköpings Universitet
Institutionen för klinisk och experimentell medicin
Avdelning för cellbiologi

Betygsnämnd:
Professor Ann-Kristin Östlund Farrants
Stockholms Universitet
Wenner-Grens Institut
Cell Biologi

Doktor Pernilla Bjerling
Uppsala Universitet
Institutionen för medicinsk biokemi och mikrobiologi

Docent Jonas Muhr
Ludwig Institute for Cancer Research
Stockholm Branch

Stockholm 2012

ABSTRACT

The linker histone H1 and the androgen receptor are two different players in the chromatin orchestra. The linker histone H1, one of the most abundant proteins in the nucleus, is located at the surface of the nucleosome but despite many important functions reported for this protein it is not as well studied as the core histones. The androgen receptor, AR, is a member of the nuclear receptor family, a conserved family of transcription factors. AR is of uttermost importance for many functions in the human body as well as a driving force behind the most common cancer form in Sweden: prostate cancer.

Paper I: Here we focus on the linker histone and the question of whether the heterogeneity of the linker histone family has a functional significance. By reconstituting individual H1 subtypes in *Xenopus* oocytes, a model system that lacks somatic linker histone, we have systematically studied their specific binding to chromatin and their effect on the chromatin structure as seen by increase in nucleosomal repeat length, NRL. We have compared linker histones that differ both in terms of origin and expression pattern as well as the ubiquitously expressed human somatic subtypes. We show that the biggest differences in terms of effect on chromatin structure are found between the coexisting human subtypes thus suggesting that H1 subtypes have different roles in the organization and function of the chromatin fiber.

Paper II: Previous studies have shown that the binding abilities of H1 are at large determined by the properties of its C-terminal domain while much less attention has been paid to the role of the N-terminal domain. Using the same assay as in Paper I we compared the binding properties of wild type H1.4 and hH1.4 devoid of its N-terminal domain (ΔN-hH1.4). We showed that the lack of N-terminal domain does not have any effect on the hH1.4 induced increase in the NRL; however, the ΔN-hH1.4 displays a drastically lower affinity for chromatin binding as compared to the wt hH1.4 and is more prone to unspecific chromatin binding. We conclude that the N-terminal domain of H1 is an important determinant of affinity and specificity of H1-chromatin interactions.

Paper III: Prostate cancer growth is regulated by AR. Antiandrogens (AR antagonists) compete with androgens for binding to AR and are thus used to stall cancer cells. However, invariably patients develop resistance to such therapy and relapse with castration-resistant prostate cancer. This motivates the creation of a second generation of AR antagonists with a more clear-cut anti AR activity. By reconstitution of the hormone regulated mouse mammary tumor virus promoter, MMTV, in *Xenopus* oocytes we previously revealed that the transcription factor FoxA1 is able to convert the glucocorticoid antagonist RU-486 to a partial agonist by presetting of the chromatin structure at the hormone-responsive enhancer. High level of FoxA1 is a negative prognostic factor in prostate cancer and we decided to evaluate the effect of the AR antagonists bicalutamide (BIC) and MDV3100 (MDV) on transcriptional outcome of AR-dependent MMTV promoter in the context of FoxA1. Here we show that both antagonists, upon binding to AR, can translocate the AR-ligand complex to the nucleus, albeit with reduced efficiency for MDV. While in the nucleus both AR-antagonist complexes have the potential to bind sequence specifically to the hormone response elements, HREs, *in vivo*. The DNA binding is strongly enhanced by co-expression of FoxA1 that makes the HREs more accessible for AR binding. In this context BIC antiandrogenic ability is seriously compromised whereas MDV shows a more persistent antagonistic activity. We believe that these findings may be of clinical relevance.

ISBN 978-91-7457-767-9