Novel regulators of prostate cancer stem cells and tumor aggressiveness

Zoni, E.

8

SUMMARY

In the past decade it became increasingly clear that tumor heterogeneity represents one of the major problems for cancer treatment, also in prostate cancer. The identification of the molecular properties of these highly aggressive cells (Cancer Stem Cells, CSCs) dispersed within the tumor represents a challenge for the identification of new efficient therapies. In most of the cases, current treatments are indeed successful in eradicating the primary tumor. However, the clinical evidence of relapse and the occurrence of therapy resistance, suggest the presence of subpopulation of cells within the tumor, that can survive such treatments and can perpetuate the cancer.

In **Chapter 1** we provide an overview of the general properties of cancer, with particular attention at the tumor heterogeneity. In this chapter we discuss the problem of prostate cancer and uncover specific aspects of tumor initiation and progression. The current diagnostic strategies are discussed and current therapeutic options for both localized and advanced disease are also addressed. Additionally we provide an overview about the molecular properties of prostate cancer stem cells and discuss the molecular pathways involved in prostate cancer progression and bone metastasis formation, with particular focus on the role of microRNAs (miR).

In **Chapter 2** we investigated the reciprocal established miR/gene interactions among the Notch, Wnt and TGF-β signaling pathways. With this approach, we identified a validated miR signature that is common to these three key signaling pathways in prostate cancer progression and bone metastasis formation. Our analysis support the cross-talk between TGF-β, Wnt and Notch signaling and their regulatory role during the process of epithelial to mesenchymal transition (EMT).

In **Chapter 3**, miR-25 was identified as a key regulator of invasion and metastasis in human prostate cancer stem cells in vitro and in vivo. The expression of miR-25 steadily and consistently increases during epithelial differentiation in the human benign prostate hyperplasia (BPH) and prostate cancers. Forced miR-25 expression in the $ALDH^{high}$ subpopulation of highly tumorigenic and metastatic prostate cancer cells strongly reduced their invasive ability. Our research led to the identification of αv- and α6-integrins as direct target genes of miR-25. Furthermore, we found that forced miR-25 overexpression in osteotropic human prostate cancer cells attenuated extravasation and subsequent metastatic colonization in vivo.

In **Chapter 4** we provide a follow-up study about the role of miR-25 in the maintenance of aggressive behaviour of prostate cancer cells. The acquisition of an invasive phenotype is key to cancer progression and characterized by enhanced motility and migration. In prostate cancer, a mesenchymal and migratory phenotype is induced by TGF-β. We found that overexpression of miR-25 abolished the TGF-β-induced migration.

In addition, we identified DACT1 as a putative miR-25 target gene. DACT1 appears to modulate the reciprocal interaction of canonical and non-canonical WNT/PCP signaling that represents one of the key regulatory pathways involved in the acquisition and maintenance of a migratory phenotype.

As previously discussed, one of the key signaling pathways involved in prostate cancer is the Notch signaling network. Notch signaling is a developmental pathway that has been shown to be involved in both prostate cancer initiation and progression, as well as in bone metastasis formation.

In **Chapter 5** we found that BMP9 supports the growth of prostate cancer and induces indirectly Notch signaling pathway activity. BMP9 expression correlates with poor prognosis and BMP9 signaling can be inhibited by administration of soluble chimeric protein (ALK1Fc), which is capable of retarding tumor growth in an orthotopic prostate cancer model in vivo.

Together these findings suggest that ALK1Fc might represent an interesting molecule for prostate cancer treatment.

Finally, in **Chapter 6**, Cripto and Grp78 have been identified as novel proteins that are involved in the formation of prostate cancer bone metastases. Knockdown of Cripto and Grp78 in human prostate cancer cells reduced extravasation of these cells when inoculated into the circulation of zebrafish embryos. Moreover, Cripto knock-down diminished bone metastasis formation in a preclinical mouse xenograft model. In line with these observations elevated Cripto expression was detected in clinical bone metastasis samples isolated from Castration Resistant Prostate Cancer (CRPC) patients.

The results of this thesis are discussed in **Chapter 7**, where we evaluated and analysed the clinical relevance and the possible therapeutic opportunities of our findings. Collectively, the studies described in this thesis have increased our insights into the molecular properties of highly metastatic and tumorigenic $ALDH^{high}$ prostate cancer stem-like cells and provided new targets for possible diagnostic and therapeutic applications.