ABSTRACT

MicroRNAs are post transcriptional gene regulators that play a critical role in normal as well as disease associated cellular processes. Research in the past decade has shown extensive association between deregulation of specific miRNA levels and cancer. miR-191 is one such microRNA that was found to be abnormally expressed in more than twenty different cancers and various other diseases. However, the functional relevance of altered miR-191 levels remains little studied.

In this thesis, we aimed to understand the basics behind aberrant regulation of miR-191 and to elucidate its functional domain in breast cancer. This study reveals miR-191 as an estrogen-inducible onco-miR in breast cancer, which promotes several hallmarks of cancer including enhanced cell proliferation, migration, chemoresistance and survival in tumor microenvironment. miR-191 is a direct estrogen receptor (ER) target and our results suggest existence of a positive regulatory feedback loop. Further, we show that the ER coactivator MED1 plays an important role in the transcriptional regulation of miR-191. We show miR-191 as a critical mediator of estrogen-mediated cell proliferation. Investigations of mechanistic details of miR-191 functions identify several cancer-related genes like BDNF, CDK6 and SATB1 as miR-191 targets. miR-191 and SATB1 show inverse correlation of expression. miR-191 mediated enhanced cell proliferation and migration are partly dependent on targeted downregulation of SATB1. Further, functional validation of estrogen:miR-191:SATB1 link suggests a cascade initiated by estrogen that induces miR-191 in ER-dependent manner to target SATB1, a global chromatin remodeler, thereby contributing to estrogen-specific gene signature to regulate genes like ANXA1, PIWIL2, CASP4, ESR1/ESR2, PLAC1 and SOCS2 involved in breast cancer progression and migration. Overall, the identification of estrogen/ER/miR-191/SATB1 cascade seems to be a significant pathway in estrogen signaling in breast cancer with miR-191 as oncogenic player.
We also explored the influence of tumor microenvironment on the function and regulation of miR-191. We found that miR-191 is a hypoxia inducible microRNA in a HIF (hypoxia inducible factor)-dependent manner and suggest existence of miR-191/HIF positive feedback loop. miR-191 overexpressing cells showed better proliferation, migration and survival under hypoxia as compared to the control cells while anti-miR-191 treated cells showed the opposite. We further established that miR-191 is a critical regulator of TGFβ-signaling and promotes cell migration by regulating TGFβ2 expression under hypoxia. The levels of TGFβ2 mRNA were found to be significantly higher in miR-191 overexpressing cells as compared to the control in 2D and 3D breast tumor models while vice versa was seen on miR-191 inhibition. The existence of both miR-191:TGFβ2 and miR-191:HuR interactions have been shown to control TGFβ2 levels under hypoxia. Notably, the levels of several HIF and TGFβ pathway genes (like VEGFA, CA9, SMAD3, EGFR, ERBB4, CXCR4, CTGF, & BMP4) were found to be higher in miR-191 overexpressing cells. Lastly, anti-miR-191 treatment given to breast tumor spheroids leads to a drastic reduction in spheroid tumor volume.

Altogether, our results suggest a critical role of miR-191 in both estrogen and hypoxia-induced cancer progression and suggest that miR-191 inhibition may offer a novel therapy for breast tumors.