A Novel Role for GSK3β as a Modulator of Drosha Microprocessor Activity and MicroRNA Biogenesis

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Background and Methods

MiRs are dysregulated in cancer, acting as tumour suppressors or oncogenes. Biogenesis of miRs is a stringently controlled and remarkably complex pathway, about which much remains to be learnt. It is vital to fully understand regulation of this process, and its perturbation in cancer, in order to exploit miRs as an 'untapped' repository of disease biomarkers and therapeutic targets. GSK3β is a serine/threonine protein kinase that plays a key role in signal transduction during processes such as cell cycle progression, proliferation and inflammation through phosphorylation of target proteins, and shows altered activity in a number of cancers, including prostate. We hypothesised that GSK3β could link pro-survival signalling pathways and miR biogenesis, and examined effects of GSK3β on Drosha's essential ribonuclease activity and its accessory proteins within the Microprocessor complex using a combination of reporter assays, qRT-PCR, *in vitro* pri-miR processing assays, *in vitro* kinase assays, mutagenesis studies, IP and RNA-IP following treatment with a small-molecule GSK3β inhibitor or transfection of constitutively-active or dominant-negative GSK3β expression constructs, in kidney and prostate cancer cells.

Results

Our data identify GSK3β as an important modulator of miR biogenesis at Microprocessor level. Repression of GSK3β activity reduces Drosha activity towards pri-miRs, leading to accumulation of unprocessed pri-miRs and reduction of pre-miRs and mature miRs without altering levels or cellular localisation of miR biogenesis proteins. Conversely, GSK3β activation increases Drosha activity and pre-miR and mature miR accumulation. GSK3β achieves this through promoting Drosha:cofactor and Drosha:pri-miR interactions: it binds to DGCR8 and p72 in the Microprocessor, an effect dependent upon presence of RNA. Indeed, GSK3β itself can immunoprecipitate pri-miRs, suggesting possible RNA-binding capacity. Kinase assays identify the mechanism for GSK3β-enhanced Drosha activity (which requires GSK3β nuclear localisation) as phosphorylation of Drosha at S^{300} and/or S^{302}. This was confirmed by enhanced Drosha activity and association with cofactors, and increased abundance of mature miRs, in the presence of phospho-mimic Drosha. Functional implications of GSK3β-enhanced miR biogenesis are illustrated by increased levels of GSK3β-upregulated miR targets following GSK3β inhibition.

Conclusions

These data, the first to link GSK3β with the miR cascade in humans, highlight a novel pro-biogenesis role for GSK3β in increasing miR biogenesis as a component of the Microprocessor complex with wide-ranging functional consequences. This has implications for GSK3β as a drug target in prostate cancer and other pathologies.

Conflicts of Interest: None

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