Mer Tyrosine Kinase Stimulated Disseminated Prostate Cancer Cellular Dormancy Escape

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Background: Many prostate cancer (PCa) recurrences are thought to be due to dormancy escape of disseminated tumor cells (DTCs). We previously found a role of the TAM family of receptor tyrosine kinases TYRO3, AXL and MERTK in PCa dormancy regulation. However, the mechanism and contributions of the individual TAM receptors is largely unknown. We systematically examined the contribution of each of the TAM receptors to PCa dormancy regulation by evaluating changes in MAP kinases, cell cycle regulators, transcription factors, and histone H3 modifications *in vitro* and confirmed the findings in mouse models.
Methods: Stable shRNA knockdowns of each of the TAM receptors or a control shRNA were created in Du145, PC3 and C4-2B PCa cells also expressing GFP and luciferase. MERTK was also knocked down by siRNA. Western blotting was performed for phosphorylated and total Erk1/2, phosphorylated and total p38, p27, SOX2, BACT1 and GAPDH. qRT-PCR with Taqman probes was conducted for NR2F1, SOX2, NANOG, and NR2F1. Cell cycle analysis was performed by flow cytometry with an antibody to incorporated BrdU and total DNA labeling with 7-AAD. Global histone H3 tri-methylation at lysine 9 and 27 was evaluated with flow cytometry as well. For *in vivo* studies, SCID mice were injected in the left ventricle with control or shRNA MERTK PC3 or Du145 cells and time to tumor formation was tracked with bioluminescence imaging. Metastasis free survival was evaluated with Kaplan-Meier analysis.
Results: Knockdown of MERTK, but not AXL or TYRO3 by shRNA in PC3 cells induced a decreased ratio of P-Erk1/2 to P-p38, increased expression of p27, NR2F1, SOX2, and NANOG, induced higher levels of histone H3K9me3 and H3K27me3, and induced a G1/G0 arrest - all of which were previously associated with dormancy in other cancers. MERTK knockdown with siRNA in PC3 and C4-2B cells also decreased expression of *SOX2*, *NR2F1* and *NANOG*. Most importantly, knockdown of MERTK in PC3 cells increased metastasis free survival after intra-cardiac injection. With the Du145 cell line, MERTK knockdown did not significantly increase metastasis free survival, but did decrease cranial metastases. MERTK knockdown also failed to inhibit PCa growth *in vitro* and subcutaneous growth *in vivo*, which suggests that MERTK has specificity for dormancy regulation or requires a signal from the PCa microenvironment. The effects of MERTK on the cell cycle and histone methylation were reversed by p38 inhibitor SB203580, which indicates the importance of MAP kinases for MERTK dormancy regulation.
Conclusions: Overall, this study shows that MERTK stimulates PCa dormancy escape through a MAP kinase dependent mechanism, and also produces the expected dormancy associated changes in p27, pluripotency transcription factors, and histone methylation. Therefore, MERTK stimulates PCa dormancy escape through intracellular pathways identified in other cancers.

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