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TITLE

Functional Role of Src Family Kinases under Hypoxia in Prostate Cancer Cells

HYPOTHESIS:

Hypoxia may activate a certain individual member in Src Family Kinases (SFKs) that contributes to hypoxia-mediated cell functions.

BACKGROUND/AIMS:

Metastasis is the major reason of disease progression and poor prognosis in prostate cancer. Src family kinases (SFKs), including c-Src, Lyn and Fyn, are non-receptor tyrosine kinases that have been shown to play an essential role in local invasion, castration resistance, and metastasis in prostate cancer. Hypoxia, a typical hallmark of solid malignancies, is able to promote metastasis-associated functions by activating various signaling molecules, including SFKs. However, whether all SFK members are upregulated by hypoxia is unclear. The current project will be investigated with three aims: Aim 1, to detect the effects of hypoxia on cell functions and SFK activities; Aim 2, to identify the individual SFK member that is mostly activated by hypoxia; Aim 3, to evaluate treatment effects of small molecule Src inhibitors in hypoxia-mediated cell functions.

METHODS:

For hypoxic exposure, prostate cancer cells were exposed to low oxygen tensions (1% O$_2$) for varied durations (0, 2, 6, 24 h). To test cell migration, "wound-healing" assay was used by making a scratch on confluent monolayers. Cells that have migrated to the denuded area were imaged. Cell invasion was detected by seeding cells into Matrigel-coated transwell chamber and cells on the bottom membranes were counted after treatment. Clonogenic assay was performed to test cell survival. At the molecular level, SFKs’ phosphorylation in cancer cells and patient tissues was detected by Western blotting, and gene knockdown was accomplished by siRNA transfection. To detect the effects of Src inhibitors, cells were treated with saracatinib with varied concentrations for indicated time periods.

RESULTS & CONCLUSIONS

Phosphorylation of all three typical SFKs, e.g., c-Src, Lyn and Fyn, were highly expressed in prostate cancer patient tissues. At the cellular level, while short term hypoxic exposure (2-6 h) induced greater effects than prolonged hypoxia (24 h) in PC-3ML, these cellular functions were increased under hypoxia for 24 h in C4-2B cells. Further, hypoxia enhanced SFK phosphorylation in the pattern that was consistent with cell functions in both cell lines. Knockdown SRC, but not LYN or FYN, abolished hypoxia-induced invasion and p-SFK expression. Lastly, SFK inhibitor saracatinib showed stronger inhibition on functional behaviors facilitated under hypoxia than normal conditions.

Our data show that hypoxia, particularly short-term exposure, is able to enhance metastatic phenotypes by activating SFKs in prostate cancer cells. Interestingly, c-Src may be the most important signaling molecule for hypoxia-mediated behaviors. These findings have shed light on the exact SFK member that is typically activated under hypoxia at least in prostate cancer model in vitro. More importantly, SFK inhibitors are able to impair hypoxia-induced cell functions, suggesting their therapeutic potential by suppressing tumor metastasis that is driven under hypoxia in prostate cancer.