ABSTRACT

Title of the thesis: The role of Ephrin and HGF/c-MET pathway in regulating Vasculogenic mimicry in breast cancer and possible effects of phytochemicals

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The occurrence of vasculogenic mimicry (VM) and EphA2-mediated tumor progression are associated with poor prognosis in various solid tumors. Here, we aimed to investigate the prognostic implications of VM and its association with phosphorylated EphA2 and c-MET receptor in invasive carcinoma of the breast (N=124). Also, to elucidate the resistance mechanism of 5-Fluorouracil (5FU)-containing therapy in triple-negative breast cancer (TNBC) and identify potential synergistic partners to overcome this clinical challenge.The patients were stratified based on CD-31/PAS dual staining and subsequently the expression status of phospho-EphA2(S897), phospho-MET, phospho-ERK1/2 and Laminin5Y2 was analyzed by immunohistochemistry. Survival of patients was correlated within the stratified cohort. Subsequently, we used our patient follow-up data (N=135) and recurrent tumors to understand the underlying mechanism of tumor resistance, followed by confirmation in an *exvivo* model. The possible crosstalk between the c-MET and EphA2 signaling networks were evaluated in vitro by siRNA silencing and in vivo tumor induction.Furthermore, the combination regimen of 5FU and Lupeol was evaluated in MDA-MB-231 cells with furthervalidation of the in vivo TNBC syngeneic mouse model and patient-derived *exvivo* culture system. The pathologically defined VM phenotype and phospho-EphA2 (S897), phospho-MET expression status was significantly associated with lower disease-free survival (DFS) and overall survival (OS). Both the features were also found to be significantly associated with higher nodal status, poor Nottingham Prognostic Index (NPI) and were more prevalent in the TNBC group. Incidentally, there were no significant association between age of the patient, grade and size of the tumor with VM and phospho-EphA2 (S897). The effector molecules of phospho-EphA2(S897) and phospho-MET viz., phospho-ERK1/2 and Laminin 5Y2 were significantly upregulated in the VM-positive cohort. Survival analysis revealed that the VM and phospho-EphA2(S897)/phospho-MET dual-positive cohort had poorest DFS and OS. Individually, VM-positive and phospho-EphA2(S897)/phospho-MET-positive expression proved to be independent indicators of prognosis. Patient follow-up data and recurrent tumors also revealed a significantly low disease-free survival in 5FUtreated patients. This revealed an association with oncogenic activation of the c-MET and EphA2 signalingpathways. The dual silencing of c-MET and EphA2 significantly abrogated the expression of downstream moleculesand other aggressive phenotypes of TNBC cells. A similar perturbation was observed with a combination of 5FUand Lupeol. The EMT status of MDA-MB-231 cells was also significantly impaired by the combination treatment.The synergistic effects of 5FU and Lupeol on an in vivo TNBC mouse model and patient-derived *exvivo* platform further mirror the combination effects.This study evaluated tumor dependency on oncogenic EphA2 receptor regulation and VM in invasive carcinoma of the breast and their prognostic significance. Significant correlations between VM, phospho-EphA2, phospho-MET and several clinicopathologic parameters of breast cancer were found. Subsequently, the occurrence of VM, phospho-EphA2 or phospho-MET expression proved to be major contributors for poor prognosis in patients with breast cancer but their simultaneous expression failed to be an independent risk factor.Our data delineated the underlying mechanism of 5FU resistance and mechanistic crosstalk between c-MET and EphA2 signaling in TNBC, leading to leveraging the translational opportunity of combining Lupeol as adrug synergy partner with 5FU to enhance its anticancer activity against TNBC.

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