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Title: Perturbation of chemo evasive mechanism of ex-vivo and in-vitro model of breast cancer stem cells: A molecular understanding for treatment failure cases of breast cancer

Abstract

The invincible role of Breast Cancer Stem Cells (BCSCs) in the development of chemoresistance is a well-known therapeutic challenge in the treatment of Breast Cancer (BC). Chemoevasion has a close association with stem cell renewal as well as several other compounding factors of the tumour microenvironment and the complexity of this mechanism is not fully characterized. Owing to their quiescent nature and comprehensive ability to repair DNA damage, chemotherapeutics that induce DNA damage (Gemcitabine, Carboplatin) cannot target BCSC. Several markers like SOX2, OCT4, NANOG as well as MDR1 and CD44 that contribute to pluripotency, stemness and chemo-evasive properties of BCSCs has been documented to be upregulated in BCSC. Previously, the role of Kaempferol, an aglycone flavonoid in attenuating epithelial-to-mesenchymal transition (EMT) in BCSCs has been established. The thesis work was done in three different study cohorts. In one set, Kaempferol alone was efficient in attenuating viability of post-NACT TNBC through downregulation of p53, MDR1, NANOG and upregulation of Caspase 3 and cleaved Caspase 3. In the second study cohort, Kaempferol (K) alone or in combination with Verapamil (V), an inhibitor of ATP driven proton pump, attenuated NANOG-CD44-MDR1 signalling cascade, that plays important role in chemoevasion. Both K and KV was found to induce DNA damage in tumour tissues but didn't show genotoxic effect to normal breast tissue, as evident by the expression of γH2AX in ex-vivo cultured adjacent breast tumour and normal tissues. In the third study cohort, we documented that KV, under low glucose condition attenuated a panoramic set of markers that play important role in chemoevasion, through tumour hypoxia, acidosis, drug efflux. KV, through ROS (Reactive Oxygen Species) overproduction, disrupted lysosomal biogenesis and lysosome-TFEB-Ca2+ signalling, one important attributor of chemoresistance. Also, an enhancement in the production of ROS after KV treatment resulted in autophagy-mediated cell death through the upregulation of LC3-II and p62 in low glucose conditions. In addition, the in-silico study validated the findings of the ex-vivo studies performed in this thesis with a parallel outcome. Earlier report stated Verapamil to be effective in depleting intracellular reduced glutathione and thus we hypothesized that KV would serve to enhance intracellular ROS overproduction in tumour cells to induce cell death and attenuate chemoevasion pathways.

In conclusion, the ex-vivo, in-vitro as well as in-silico studies revealed that the candidate drug combination KV could effectively target several pathways regulating chemoresistance stemness apoptosis phenomenon, that were not hitherto studied in the same experimental setup and thus may be endorsed for therapeutic purposes.

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