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Title. Exploring the role of exosomes in the metabolic regulations of metastatic ovarian cancer

Abstract

Ovarian cancer (OvCa) turns as the alarming condition across the globe governed by its metastasizing capacity and disease-associated inflammation leading to increased mortality rate. The dreadful disease might be recognised as altered metabolically programed environment in which the cancer associated metastasis is controlled by several factors, among them, the extracellular vesicles i.e. exosomes, have been in the limelight in current research. Thus, this study aims to understand the molecular mechanisms that adversely influence the metastasis of the disease, leading to poor outcomes in the survival of the patients.

Metastasis involves a plethora of biomolecules actively participating in devising the tumor microenvironment (TME) for proliferation and survival of cancer cells. An extensive database mining revealed higher SIRT1 status, disorients numerous biological processes, expressing with its genomic abnormalities in OvCa, which instigated us to investigate the molecular crosstalk augmenting the process of metastasis. Further, we identified SIRT1 associated hub genes played a significant role epithelial-mesenchymal transition (EMT) and cellular signalling cascade PI3K/AKT, thereby illustrating SIRT1 as a notable target in OvCa therapy. Additionally, a comparative analysis of a small molecule inhibitor (SMI) and a phytochemical named Hyperforin by Molecular docking confirmed SIRT1 Inh III as a potent inhibitor of SIRT1 whose potency was further validated by *in-vitro* and *in-vivo* analysis.

A clinicopathological analysis of OvCa was conducted to depict the risk factors for the development of OvCa, revealing that Body Mass Index (BMI), metastasis, degree of dissemination were significantly correlated with increasing levels of the diagnostic marker, CA-125, which negatively impacted the survival of the patients. Furthermore, the expression of SIRT1 was found to be regulated by elevated expression of HIF-1α, establishing a hypoxic TME under the effect of increased release of exosomes from the tumor tissues as compared to the adjacent normal tissue, thereby driving the proliferation of cancer cells as denoted by heightened Ki-67 expression. This phenomenon is supported by the continuous supply of energy from elevated glycolytic pathway as indicated by increased deposition of glycogen in the cancer tissues. All these findings underscored the intricate interplay between all these molecules in the TME of OvCa with the idea that increasing levels of exosomes and SIRT1 expression can precise the staging of OvCa and aid clinicians in planning respective therapeutic strategies for the patients.

Upon further analyzing the sociodemographic parameters, we deduced that pain is a major symptom that prevailed in the OvCa patients, with its severity escalating significantly with

tumor differentiation. Thus, in-depth analysis of the molecular circuitry revealed COX-2 to be significantly upregulated in the poorly differentiated OvCa tissues. Additionally, SIRT1/COX-2 colocalization was also strikingly higher in the tumor tissues that reinforced the metastatic process by increasing the colocalized expression of MMP2/MMP9 and VEGF/ANGPT2. Alternatively, decreasing colocalized SIRT1/PTEN expression indicated a compromised tumor suppressive function of PTEN under the effect of increased cytokines levels. Further investigation revealed that under the interference of exosomes in reprogramming the TME, the levels of SIRT1/COX-2/IL-6 increased, but the inhibition of SIRT1 decreased their expression. Positive results were also observed with EMT markers, E-cad and Vim. Apoptotic markers, Bax and Bcl-2, along with reactive oxygen species (ROS) generation, also revealed significant differences in their expression under the impact of exogenous exosomes, which eventually got mitigated under the effect of SIRT1 Inhibitor.

The exosomal orchestration was not only limited to the cancer cells but also induced oncogenic transformation into normal cells, which was confirmed by heightened expression of SIRT1/COX-2/IL-6 axis, as well as the alterations in EMT markers (E-cad and Vim), in presence and absence of SIRT1 Inhibitor. A deeper investigation identified PI3K/AKT signaling cascade to be elevated under the effect of exosomes that diminished under the effect of SIRT1 Inhibitor. *In-vivo* analysis validated that blocking exosomal secretion in combination with metabolic inhibition could also remarkably reduce the metabolic markers (SIRT1), metastatic and EMT markers (MMP2/MMP9, VEGF/ANGPT2, E-cad/Vim/Snail, Ki-67), and glycolytic markers (GLUT1/HKII/PKM). Additionally, the mitigation of increased glycogen deposition and greater glucose uptake accompanied by increased pyruvate content, lessened lactate generation and ATP levels under the decreased activity of SIRT1 enzyme also confirmed that metabolism and metastasis are strongly intertwined in OvCa. Concisely, targeting exosome-mediated signaling by selectively inhibiting SIRT1 grants a promising therapeutic strategy to treat the disrupted metabolic-metastatic axis and improve patient survival outcomes.

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