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EZH2-H3K27me3 mediated KRT14 upregulation promotes TNBC peritoneal metastasis

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Triple-Negative Breast Cancer (TNBC) has a poor prognosis and adverse clinical outcomes among all breast cancer subtypes as there is no available targeted therapy. Overexpression of Enhancer of zeste homolog 2 (EZH2) has been shown to correlate with TNBC's poor prognosis, but the contribution of EZH2 catalytic (H3K27me3) versus non-catalytic EZH2 (NC-EZH2) function in TNBC progression remains elusive. We reveal that selective hyper-activation of functional EZH2 (H3K27me3) over NC-EZH2 alters TNBC metastatic landscape and fosters its peritoneal metastasis, particularly splenic. Instead of H3K27me3-mediated repression of gene expression; here, it promotes *KRT14* transcription by attenuating binding of repressor SP1 to its promoter. Further, KRT14 loss significantly reduces TNBC migration, invasion, and peritoneal metastasis. Consistently, human TNBC metastasis displays positive correlation between H3K27me3 and KRT14 levels. Finally, EZH2 knockdown or H3K27me3 inhibition by EPZ6438 reduces TNBC peritoneal metastasis. Altogether, our preclinical findings suggest a rationale for targeting TNBC with EZH2 inhibitors.

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According to global cancer statistics, breast cancer is the most common malignancy worldwide in women, having 2.09 million new cases diagnosed in 2018 with 0.6 million deaths[1]. Among different breast cancer subtypes, basal-like breast cancer is often clinically defined as triple-negative breast cancer (TNBC), which lacks immunohistochemistry (IHC) expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (her2/neu) according to College of American Pathologist (CAP)/ASCO guidelines[2]. It involves patients of young age and is associated with high-grade, poor-prognosis tumors[3]. TNBC poses a serious threat to clinicians due to the enormous heterogeneity of the disease and the absence of well-established molecular targets[4]. Indeed, the 90% mortality of breast cancer, including TNBC, is associated with metastasis[5–8]. Underpinning the molecular cues for TNBC metastasis and its therapeutic intervention are the hotspots of current cancer research. The concept of epigenetic reprogramming is admired as a driving force for distant organ metastasis. Importantly, Breast cancer molecular subtypes have been identified with unique chromatin architecture with diverse methylation patterns[9,10]. However, how epigenetic mechanisms exclusively regulate subtype-specific distinct transcriptional nexus still remains elusive. Epigenetic modulator Enhancer of Zeste homolog 2 (EZH2), a catalytic subunit of Polycomb Repressive Complex 2 (PRC2), promotes target genes suppression by tri-methylation of lysine 27 of histone H3 (H3K27me3)[11]. Previously, we have shown the classical gene silencing function of EZH2 where death receptors are epigenetically suppressed in cancer stem cells[12]. Notably, aberrant EZH2 expression has been associated with diverse cancers concerning both oncogenic and tumor suppression functions[13]. Besides its canonical function as a transcriptional repressor, EZH2 protein has recently been shown to perform H3K27me3 independent functions[14–18]. In support of non-canonical functions, studies have shown a hidden, partially disordered transactivation domain (TAD) in EZH2, which directly binds to the

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Loss of PERK function promotes ferroptosis by downregulating SLC7A11 (System Xc⁻) in colorectal cancer

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ABSTRACT

Ferroptosis, a genetically and biochemically distinct form of programmed cell death, is characterised by an iron-dependent accumulation of lipid peroxides. Therapy-resistant tumor cells display vulnerability toward ferroptosis. Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR) play a critical role in cancer cells to become therapy resistant. Tweaking the balance of UPR to make cancer cells susceptible to ferroptotic cell death could be an attractive therapeutic strategy. To decipher the emerging contribution of ER stress in the ferroptotic process, we observe that ferroptosis inducer RSL3 promotes UPR (PERK, ATF6, and IRE1α), along with overexpression of cystine-glutamate transporter SLC7A11 (System Xc⁻). Exploring the role of a particular UPR arm in modulating SLC7A11 expression and subsequent ferroptosis, we notice that PERK is selectively critical in inducing ferroptosis in colorectal carcinoma. PERK inhibition reduces ATF4 expression and recruitment to the promoter of *SLC7A11* and results in its downregulation. Loss of PERK function not only primes cancer cells for increased lipid peroxidation but also limits in vivo colorectal tumor growth, demonstrating active signs of ferroptotic cell death *in situ*. Further, by performing TCGA data mining and using colorectal cancer patient samples, we demonstrate that the expression of *PERK* and *SLC7A11* is positively correlated. Overall, our experimental data indicate that PERK is a negative regulator of ferroptosis and loss of PERK function sensitizes colorectal cancer cells to ferroptosis. Therefore, small molecule PERK inhibitors hold huge promise as novel therapeutics and their potential can be harnessed against the apoptosis-resistant condition.

1. Introduction

Cancer is a leading cause of death worldwide; in 2020, there were 19.3 million new cases of all types of cancer in which more than half of the patients died. Colorectal cancer is the third most commonly diagnosed cancer (10.0%) and the second leading cause of death (9.4%) worldwide in both males and females [1]. Therapy resistance is the key to tumor relapse and subsequent tumor-associated mortality. Evasion of apoptosis is one of the important hallmarks of cancer cells and mechanisms behind the same have enormous therapeutic potential in the context of current cancer research [2]. Recently, we have shown how intracellular CXCR4 protein and epigenetic modulator EZH2 promote therapy resistance, CSC properties and metastasis in colorectal and breast cancer [3–5]. Recent reports also suggest that these resistant cancer cells are vulnerable to iron-mediated cell death or 'Ferroptosis' [6,7].

As originally discovered by the Stockwell group, ferroptosis is morphologically, biochemically and genetically distinct from apoptosis, necroptosis, and autophagy and depends on intracellular iron [8,9]. Selenoprotein Glutathione peroxidase 4 (GPx4), cystine/glutamate antiporter (System Xc⁻) and enzyme Acyl-CoA synthetase long-chain family member 4 (ACSL4) are known to be the key modulators of ferroptotic process [10–12]. GPx4 is the critical enzyme that can reduce lipid hydroperoxides within biological membranes; hence ferroptosis can be induced by the treatment of small molecule GPx4 inhibitor RSL3 (Ras Selective Lethal) treatment [13,14]. SLC7A11 or System Xc⁻ is a multi-pass transmembrane protein that facilitates the export of intracellular glutamate and import of extracellular cystine in a 1:1 ratio [15].

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