Characterization of novel inhibitors for triple negative breast cancer: four needles in a haystack

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Introduction: Worldwide, breast cancer is now the most prevalent form of the disease overall[1]. In the majority of African countries, breast cancer is the most frequently diagnosed malignancy in women [1] and half of all patients will die of the disease [2]. Furthermore, it hasbeen demonstrated that the aggressive and treatment-resistant triple negative or $ER^{-}PR^{-}HER\text{-}2^{-}$ subtype is more common among Black women, necessitating the search for novel therapiesfor this form of the disease [3].

Methods: Thiazolidinone (TAL) compounds OY25 and OY29 and ferrocenyl bezoxazines (FBX) 5b and 7e were screened and characterized. Cell lines utilized included HCC70, HCC1937 and HCC1806 TNBC lines, together with MCF-7, HeLa, MCF12A, HEK293T, VERO and MEF-1 lines. A broad range of methods were employed, including the resazurin cytotoxicity assay, agarose gel electrophoresis, methylene blue and Hoescht33342 assays, *insilico* docking studies, the alkaline comet assay, western blot analysis and fluorescentmicroscopy, thermal shift assay, protein pyruvate kinase muscle 2 (PKM2) glycolytic activity assay, Hsp90 ATPase (luciferase) and topoisomerase (TOPO) II decatenation assays.

Results: We describe the characterization of two TAL hit compounds that induce a robust DNA damage response whose mechanism of action involves direct binding to DNA in the minor groove and significant downregulation of the glycolytic enzyme PKM2. The latter enzyme is overexpressed in cancer and moonlights in DNA damage repair processes via phosphorylation by ATM, causing resistance to DNA damage induction by radiation therapy [4]. Interestingly, the inhibition of PKM2 was independent of its glycolytic enzyme activity, suggesting specific inhibition of this moonlighting function and a potential dual mode of action of the hit compounds. We also describe here the analysis of two novel FBX compounds in terms of their ability to function as GHKL inhibitors. The DNA gyrase class of topoisomerases,the molecular chaperone Hsp90, histidine kinases and MutL-like DNA-mismatch-repair proteins (GHKL) superfamily share a common nucleotide binding domain called the Bergerat fold, allowing potential simultaneous targeting of multiple oncogenic proteins by GHKL inhibition [5]. Both FBX compounds were found to dock into the Bergerat fold of the GHKL proteins and were able to bind directly to DNA and cause significant DNA damage as well as protein aggregation in TNBC cells. Interestingly, while compound 7e inhibited Hsp90 ATPase activity, compound 5b caused significant inhibition of TOPO II DNA decatenation activity.

Discussion and conclusion: We demonstrate here the characterization of novel TAL and FBX compounds as DNA damage inducing agents that modulate PKM2 and Hsp90/TOPO II activity, respectively, in TNBC cells *in vitro*.

Keywords: triple negative breast cancer, thiazolidinones, pyruvate kinase muscle 2, ferrocenyl benzoxazines, Hsp90, topoisomerase II.

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