Unfolded Protein Response activation in pancreatic cancer cells is synthetic lethal with ferropotosis

Gobbi M.[1], Zerbato B. [1], Ludwig T.[2], Wegner A.[2], Chiaradonna F.[1]
E-mail: m.gobbi5@campus.unimib.it
[1] University of Milano Bicocca, Department of Biotechnology and Biosciences, Piazza della Scienza 2, 20126 Milan, Italy
[2] Department of Bioinformatics & Biochemistry, Rebenring 56, 38106 Braunschweig, Germany

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Abstract:
FR054, a specific inhibitor of Hexosamine biosynthetic pathway (HBP), it has been shown to exert anticancer activity through activation of Unfolded Protein Response (UPR), generation of reactive oxygen species (ROS), inhibition of EGFR signalling and inducing apoptosis. To further detail the transcriptional consequence of HBP inhibition, we performed a transcriptional analysis of pancreatic cancer cells (PCs), namely MiaPaca2 and BxPC3, 48h-treated with FR054. Differentially expressed genes (DEG) were analyzed with different bioinformatics tools. As expected, transcriptional data indicated activation of processes related to protein folding such as intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, confirming the tight relation between HBP role in protein N-glycosylation and Unfolded protein Response (UPR) activation. Strikingly, transcriptional data indicated also a concurrently enrichment of terms associated with phospholipids remodelling and Ferroptosis. Worth of note Ferropotosis is characterized by the accumulation of lipid peroxides due to the failure of glutathione-dependent antioxidant defences. However, analysis of the expression level of ferropototic genes indicated that UPR activation was able to induce an antioxidant and anti-ferropototic response. In particular genes involved in glutathione synthesis such as the cystine/glutamate antiporter (*SLC7A11* and *SLC3A2*), glutamine and cysteine metabolism (*GCLC*, *GCLM*, *GOT1*, *CTH*) and iron transport and storage (*TFRC*, *FTH1*, *FTL*) were all significantly up-regulated. Given that, we tested if inhibition of these antioxidant responses could create conditional vulnerability with FR054 in PCs. Cell growth and apoptotic analysis of cancer cells treated with FR054 alone or in combination with Erastin, an SLC7A11 inhibitor able to induce Ferropotosis, indicated that the two drugs had a synergistic effect on both cell proliferation and cell death since the combined treatment induced a completely block of cell proliferation and enhanced significantly cancer cell death as compared to untreated or single drug treated cells. Importantly metabolomics data indicates that FR054 changes glutamine metabolism favouring glutamate release, confirming the role of SLC7A11 in cancer ability to cope UPR stress enhancing GSH synthesis. Altogether these findings indicate that Ferroptosis in association with HBP inhibition might be a novel anticancer mechanism to be exploited as therapy.