Influence of oncometabolites in the response to DNA damage

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Mutations in genes encoding Krebs cycle enzymes, such as succinate dehydrogenase (SDH) and fumarate hydratase (FH), result in the acucumulation of their respective metabolites, succinate and fumarate. This accumulation produces a deregulation of the energetic metabolism, and leads to the development of a tumoural phenotype. Besides, through the alteration of chromatin structure, by inhibiting histone and DNA demethylases, these metabolites might modify DNA repair and, then, influence cancer treatments, like chemotherapy and radiotherapy. With this in mind, the general aim of this work is to study the impact of these molecules on DNA damage responses (DDR), after treatment with different genotoxic agents.

PC12 cells, from a rat pheochromocytoma of the adrenal medule, were used to study the influence of succinate and fumarate in the response to hydrogen peroxide, and human GM04312 cells (*XPA* mutant) to cisplatin induced DNA damage. Presence of the proteins SDHA, SDHB and FH were determined by immunofluorescence in PC12 cells. The influence of metabolites was studied with 1 h pretreatments, using 1-5 mM concentrations (lower than the respective IC50), followed by treatments with 200 μM H_2O_2 for 10 min, or with 20 μM cisplatin for 3 h. Methylated and/or unmethylated metabolites were used to check the effect of the cell-entering pathway. DDR were studied analysing cell viability, cell cycle progression, apoptosis, and DNA damage.

Results show that these metabolites, at the analyzed conditions and concentrations, were not toxic, and did not modify cell cycle progression nor apoptosis; however, they slightly increased the detected DNA damage, probably due to an impaired repair of spontaneous DNA damage. In response to the genotoxic agents, although no effects on viability or cell cycle progression were detected, influences of these metabolites were detected on apoptosis and induced DNA damage. Furthermore, differences were found between the methylated and unmethylated metabolites, both for DNA damage and apoptosis. In general, fumarate and methylfumarate increased the genotoxin-induced DNA damage, whereas succinate and methylsuccinate did not. Moreover, succinate and methylsuccinate showed different effects, problably related with their cell-entering pathway.