INDOMETHACIN INHIBITS PROLIFERATION IN CELLS HARBOURING PIK3CA MUTATIONS

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PIK3CA is one of the most commonly mutated oncogenes in human cancers (Samuels & Waldam, 2010). Specific *PIK3CA* mutations confer constitutive activation of PI3K, which promotes cell proliferation and survival via intracellular kinase signalling cascades induction. Tumours with highest frequencies of PIK3CA mutations include colon, breast and endometrial tumours (Samuels & Waldam, 2010). *Indomethacin*, a non-steroidal anti-inflammatory drug (NSAID), is known to inhibit cell proliferation (Bernardi et al., 2006). We have previously evidenced that, in a cell-based proliferation assay, non-transformed epithelial cell lines of breast (hTERT-HME1), in which mutations in the EGFR pathway were introduced utilizing adeno-associated-viral (AAV) mediated homologous recombination, the isogenic clone harbouring *PIK3CA* H1047R mutation was more sensitive to indomethacin treatment (Di Nicolantonio et al., 2008). This effect seemed to be indomethacin-related as other NSAIDs, such as celecoxib, acetilsalicylic acid and meloxicam, were not able to selectively inhibit *PIK3CA* H1047R mutated cells proliferation. Based on the above reported observation, we performed experiments trying to dissect the mechanism of indomethacin mediated inhibition of proliferation. With this aim we used hTERT-HME1 wild type or isogenic clones harbouring different mutations, specifically *PIK3CA* H1047R, *EGFR* delE746-A750, *KRAS* G13D and *BRAF* V600E. The cells were pre-treated with a dual ATP-competitive PI3K and mTOR inhibitor (BEZ235) before observing the effects of indomethacin on cell viability, measured by the ATP bioluminescent assay. The pre-treatment of cells with BEZ235 reduced the inhibition of proliferation mediated by indomethacin mainly in *PIK3CA* H1047R, suggesting a direct role of PI3K pathway on this effect. Western blot analysis of PI3K-Akt and MAPKs pathways evidenced that both are modulated by indomethacin treatment. These results were confirmed utilising colorectal cancer cell lines harbouring mutations in the EGFR pathway as those present in the isogenic clones. In parallel, the effect of the indomethacin on PI3K *wild type* and PI3K-H1047R mutated protein was investigated using *structural*- and *ligand*-based drug design techniques. In the first approach, a modelling study was dedicated to investigate the binding differences in the two different PI3K related proteins. In the second one, the indomethacin related analogues were designed in order to loose COX-related properties and improve the effect on the PI3K-H1047R protein. Our results suggest that indomethacin could be able to interact in a more selective way with PI3K H1047R than with PI3K *wild type.*