Celecoxib Targets CML Blasts Proliferation in a COX-2 independent manner

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Beyond its use as anti-inflammatory drug, celecoxib (CELE), the most characterized among the inhibitors of cyclooxigenase 2 (COX-2) is known to exert an antiproliferative action in many models of solid tumours (in particular colon cancers carcinomas). However, its effects on 'liquid' tumours have not been fully elucidated. In order to fill this void in knowledge, we assessed whether CELE suppresses the proliferation also of human Philadelphia-positive (Ph+) chronic myelogenous leukaemia blasts, and, in addiction, whether these effects are COX-2-dependent or independent.
By using human cell lines and leukemic blasts from patients we demonstrated the cytostatic/cytoxic action of CELE trough specific assays (MTT and 'clonogenic' assays on methyl-cellulose). Importantly these effects appeared as unrelated to COX-2 inhibition since this enzyme was not expressed by leukemia blast (as documented by immnuoblots and rt-PCR) and rofecoxib (another selective inhibitor of COX-2) was not able to recapitulate the action of CELE. Assessment of DNA content and integrity through flow cytometry and fluorescence microscopy also allow to demonstrate a consistent increase in the G1-phase cell subset as well as features of apoptotic degradation only in samples treated with CELE (25 μM). Molecular characterization of CELE mechanism of action by means of biochemical and genetic approaches allowed us to demonstrate the prompt activation of AMP-activated protein kinase (AMPK) and the consequent inhibition of both mTOR complexes (i.g. mTORC1 and 2). Moreover we demonstrated the such an impairment of the mTOR pathway is responsible for a GSK-3β-dependent down-regulation of β-catenin likely to give a contribution to the anti-leukemic effect of CELE. Moreover, we demonstrated the ability of CELE to affect clonogenicity of imatinib-resistant CML blasts (the T315I mutant of BCR/ABL) and to synergize with imatinib when administered to TKI-responsive cells. Of importance from a therapeutic point of view, we confirmed the anti-proliferative effects of CELE in CML CD34+ cells isolated from three chronic phase patients but not in normal CD34+ progenitors, a result that strongly supports its selectivity toward Ph+ hemopoietic cells.
Overall our observations strongly propose CELE as lead compound for targeting AMPK activation in view of intercepting mTOR and β-catenin pathways, which are known to play an important role in the self-renewal of the leukemic stem cell compartment and therefore in the acquisition of resistance to TKI-based therapies.