Deregulation of ABC transporters in a CML resistant cell model

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Chronic Myeloid Leukaemia (CML) is characterized by the uncontrolled clonal proliferation of totipotent stem cells or already oriented in the myeloid sense, which results in depression of the regular haematopoiesis. CML accounts for 15% of adult leukaemia and has an incidence of 1 to 2 cases per 100.000 adults [1]. The cytogenetic abnormality characterising CML patients is the Philadelphia chromosome (Ph), a translocation of chromosomes 9 and 22 (t(9;22)), resulting in the BCR-ABL fusion protein. BCR-ABL gene encodes for an abnormal tyrosine-kinase protein that promotes the neoplastic transformation of the cells harbouring the Ph chromosome [2]. Imatinib mesylate (IM) acts blocking the kinase activity of that protein. IM was the first available BCR/ABL-targeted therapy, producing complete cytogenetic responses in 70-85% of CML patients in the early chronic phase (CP), in which the majority of the diagnosis occurs. IM is an orally administered drug that competes with the adenosine triphosphate (ATP) binding site on ABL, leading to tyrosine phosphorylation inhibition of the proteins involved in BCR-ABL signal transduction, resulting in apoptosis of cancer cells [3].

Despite the remarkable efficacy of IM, it has been observed a high inter-individual variability, depending on several possible factors, including point mutations or inter-individual adaptation processes. Among the most significative mechanisms of resistance, we find the over-expression of the ABC transporters. These membrane proteins act as "pumps", carrying the anticancer drug out of the cell itself, and preventing its cytotoxic action [4]. To explore in detail this option, we have set up a human CML cell model, K562, treating them with increasing concentrations of IM (from 0.05 μM to 3 μM). RNA was extracted at each change of concentration and reverse transcribed to cDNA. The cDNA was finally loaded to a 384 wells micro-fluidic cards, the Human ABC Transporter Array, which allow to perform simultaneously 384 Real-Time PCR reactions, using the TaqMan technology (Applied Biosystem). After Ct normalization, the samples were compared to determine any gene expression alterations associated with the development of IM resistance. We observed that, at the highest concentration (3 μM), the majority of transporters presented deregulated expression and, in particular, six genes -ABCG1, ABCC3, ABCB5, ABCA4, ABCC13, and ABCD2- were over-expressed compared to untreated cells.