Construction of a novel bioluminescence bacterial biosensor for real-time monitoring of cytotoxic drugs activity

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A bacterial lux reporter has been developed to measure the activity of cytosine arabinoside (Ara-C), a synthetic pyrimidine nucleoside analogue, that is the mainstay of treatment for acute myeloid leukaemia (AML). Since Ara-C has to be taken up and converted by the cell to its active derivative Ara-CTP, resistance to chemotherapy is common and 30% of patients fail to achieve remission. Current methods for measuring AML response to Ara-C are complicated, expensive and require lengthy incubation times. The bioluminescent reporter was constructed by the addition of constitutively expressed lux genes to E.coli strain (SO5110) defective in its pyrimidine salvage pathway. This strain is sensitive to Ara-C due to the expression of the human deoxycytidine kinase gene (dCK) under the control of the lac promoter. In the presence of IPTG, the growth of E.coli SO5110 after 4-5 hrs was reduced by 48% when 20 µM Ara-C was added. E.coli SO5110 was made constitutively bioluminescent when transformed with pAL2 carrying the modified lux ABCDE operon from Photorhabdus luminescens. The activity of Ara-C on E. coli SO5110 (pAL2) was determined by monitoring O.D.600 and light output at concentrations of 25, 50, 75 and 100 µM (clinical dose). As previously reported, the effect of Ara-C was inhibitory to culture growth; however, the bioluminescence of E.coli SO5110 (pAL2) showed an increase of between 74 and 172 %, in a concentration-dependent manner. The biosensor construct did not respond to Ara-C in the absence of IPTG, indicating that the activity of the human dCK gene is essential, and that the biosensor is able to detect Ara-CTP. It therefore has potential for use as a biosensor within human AML cell samples and for rapid and non-invasive screening of cancer cell sensitivity to nucleoside analogues.