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ROLE OF JAK-STAT SIGNALING IN SORAFENIB RESISTANT HCT-116 CELLS

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ABSTRACT : Sorafenib is a small molecule, multi-kinase inhibitor used as an anti-cancer drug for treatment of Liver and Kidney cancers. It is reported to be in phase 2 clinical trials for colorectal cancer (CRC). This drug was reported to inhibit both surface associated and intracellular kinases such as Vascular Endothelial Growth Factor Receptor (VEGFR), Platelet-Derived Growth Factor Receptor (PDGFR), Fibroblast Growth Factor Receptor (FGFR), RAF, BRAF etc. This was shown to be effective against both mutant and wild type BRAF. There are some preliminary clinical reports of patients showing resistance to this drug on long-term usage. Our study aims at developing a resistant cell line model for the above drug and to understand the pathways and mechanisms involved in resistance development. Resistant model of HCT-116 cell line was developed by continuously culturing parental cell line in the presence of the drug. The IC_{50} values were compared between the parental cells and the cells exposed to drug continuously.

BCL2 an anti-apoptotic protein was seen overexpressed in resistant cells in contrast to decreased expression in parental cells supporting the development of resistance. Further the multidrug resistance proteins MDR1 and ABCG-2 are highly up regulated in resistance model reconfirming the resistance development. This study aims at understanding how the JAK-STAT signaling pathway is modulated during drug resistance by expression profiling of molecules from this pathway by real time PCR. In this study, we found that JAK-STAT pathway is suppressed in the parental cells when treated with the sorafenib, but found actively expressed in resistant model. JAK-STAT signaling pathway is known to be activated in cancers and helps in regular proliferation of cancer cells. But as noticed from the above results upon drug treatment in parental cells this pathway get suppressed thereby leading to cell death. The active involvement of JAK-STAT pathway, which supports proliferation, was seen over expressed in drug resistance model.

Key words : Colorectal cancer, Sorafenib, Jak-Stat pathway, resistance model, kinase and HCT-116 cell line.

INTRODUCTION

In recent few decades the rate of incidence of cancer is increasing very fast and after heart disease it is 2nd most leading cause of deaths worldwide (WHO media center Fact Sheet-2015). According to GLOBOCAN 2012 an estimated 14.1 million new cancer cases, 8.8 million cancer related deaths and 32.6 million people living with cancer worldwide (CDC, NCSHs and Fast Stats - 2015). Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer (1.4 million i.e. 9.7%) and 4th leading cause of cancer related death (Ferlay *et al*, 2012). Surgery (Hohenberger *et al*, 2009; Schmiegel *et al*, 2004; Sehgal *et al*, 2014), radiotherapy (Baskar *et al*, 2012; Begg *et al*, 2011; Jackson *et al*, 2009) and chemotherapy (Bleiberg *et al*,1998; Fujita *et al*, 2015; Kurniali *et al*, 2010; Liu *et al*, 2000; Longley *et al*, 2003; Matherly *et al*, 1990; Miwa *et al*, 1998; Park *et al*, 1988; Wohlhueter *et al,* 1980) are being used for most of the cancer treatment but the limitations associated with these are tumor recurrence, low efficiency and tumor acquiring resistance against all the therapies (Li *et al*, 2016; Zheng *et al*, 2015; Duldulao *et al*, 2013; Keppler *et al*, 2015; Marin *et al*, 2012). Kinases are a large group of enzymes involved in mediating phosphate group transfer from high-energy molecules such ATP/GTP to the substrate results into change in the activity of the substrate ("Kinase". The Free Dictionary.com). Now in recent years the pharmaceutical companies shifted towards designing small molecule inhibitors against kinases.

Sorafenib is the 1st small molecule based multi-kinase inhibitor successfully developed and approved for the treatment of renal cell carcinoma (FDA Approval letter for use of sorafenib in advanced renal cancer. December 2005) and hepatocellular carcinoma (FDA Approval letter for use of sorafenib in inoperable hepatocellular carcinoma November 2007). *In vitro* biochemical assays confirmed that sorafenib is a potent inhibitor of Raf1 kinase (IC_{50} of 6 nM) (Wilhelm *et al*, 2004). Sorafenib has also reported to inhibit wild-type B-Raf and oncogenic *B-Raf* V600E serine/threonine kinases, receptor tyrosine kinases (RTKs) (VEGFRs 1/2/3, PDGFRβ, FGFR1, c-Kit, Flt-3