Chapter 5
Conclusion
Despite significant progress in early detection and systemic treatment of the cancers, most of the available therapies fail because tumor cells acquire resistance against these therapies. Sorafenib and Regorafenib are multi-kinase inhibitors being used for the treatment of advanced stages of hepatocellular cancer, renal cell carcinoma and colorectal cancer. There are a few reported cases of drug resistance development against Sorafenib till date. But Regorafenib being structurally similar might also have the chance of developing resistance so we intend to develop a resistance cell line model against these drugs in colorectal cell lines HCT-116 and HT-29 cells. In this study the cells were continuously exposed to the drug and were made resistant to the drug. The resistance development was further confirmed by increased drug tolerance by these cells i.e. elevated IC$_{50}$ concentrations of the drug exposed cells. Also the increased expression of BCL2 and decreased expression of pro-apoptotic molecules like caspases, BAD, BAX, PARP etc further supported the resistance development. The increased expression of MDR1 and ABCG2 genes, which are reported to play a major role in drug resistance were also up-regulated in our resistant cell line. These results further points towards resistance development in these cells against Regorafenib and Sorafenib. Looking the higher fold change of 2.5 folds increase in IC$_{50}$ concentrations in regorafenib resistant HCT-116 cells are selected for further analysis.

Various signaling pathways that are upregulated in cancers were studied to see the alterations in these pathways during resistance development. JAK-STAT signaling is involved in various biological processes such as immunity, proliferation, differentiation and oncogenesis (Ishihara K. et al., 2002). Several studies reviewed that JAK-STAT pathway gets activated in Sorafenib-resistant HCC cells. In this study we found the elevated expression of JAK1, JAK2, STAT3 and its target MCL1 and cMYC genes indicating that the JAK-STAT pathway participates in the acquired resistance to Sorafenib and Regorafenib. Still it is unclear how JAK-STAT signaling gets activated in the resistant tumor cells and how exactly it helps in acquiring resistance.
The PI3K is activated by G protein-coupled receptors and tyrosine kinase receptors (Leevers SJ et al., 1999). PI3-kinases involved in various basic cellular processes, such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. Aberrant activation or up-regulation of PI3K-AKT signaling has been suggested to be important mediators of drug-resistance (Long et al., 2011, Lin L. et al., 2014). In addition, as a member of the AKT pathway, the mechanistic target of Rapamycin (mTOR) is also activated in the drug resistant tumors (Baskar R. et al., 2012). In this study we identified that elevated expression of PI3K and AKT at both mRNA and protein level. We also found up-regulation of mTOR signaling and its associated target genes in the drug resistant cells. This result indicates its participation in the acquiring resistance against Sorafenib and Regorafenib.

Raf-Mek-Erk is another intracellular signaling cascade, which is activated through receptor tyrosin kinase activity. Aberrant activation of Ras-Raf-Mek-Erk signaling results in initiation and progression of human cancers. In this study we observed that elevated expression of RAF1 and ERK 1/2 genes in the Sorafenib and Regorafenib resistant models indicates its participation in acquiring resistance. Further studies to understand the role of these singling pathways in acquiring resistance and what are the up-stream molecules that activate these pathways needs to be carried out.

Further to understand the alterations in the complete cellular metabolism in the cell during drug resistance development we have performed proteomic studies by Mass-Spectrometry. In this study we could identify around 232 differentially expressed proteins from that 114 proteins are up-regulated and 118 proteins are down-regulated. Up-regulated proteins are involved in various pathways like mRNA polyadenylation, embryonic brain development, histone methylation, negative regulation of neuron differentiation, negative regulation of phosphatase activity, notch receptor processing, integrin mediated signaling, notch signaling pathway, cell adhesion and protein binding etc. Many tumor promoter genes were found over-expressed such as CBX8, PI3K, ZNF267, APP, ADAM10, JAG1, CCND1, ADAMTSL1, CALR and RAD21 etc.

Whereas the down-regulated proteins were involved in histone demethylation activity, neuromuscular junction, cytoskeleton development, protein sumoylation
sperm motility, ATPase activity, zinc ion binding etc. We also found various tumor suppressor genes were down-regulated such as BRCA1, PARP15 etc.

The mi-RNA profiling showed about 700 differentially expressed micro RNA which are linked to all the pathways like JAK-STAT, PI3K, AKT, Notch, mTOR, Wnt, Cell adhesion signaling, Hedge-hog signaling etc. The actual involvement of all these pathways and cross talk between these pathways needs to be studied in detailed.

These are the first resistant cell line model created against Regorafenib and Sorafenib. This being the first study gives us the basic understanding about drug resistance development. But the exact pathways altered and the activation of other pathways in response to these alterations needs further evaluation. These in-vitro results need further evaluation in vivo models and clinical samples so as to improve the therapeutic strategies against drug resistant colorectal cancers.

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