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In Silico Analysis of Differentially Expressed Genes in Colorectal Carcinoma

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Abstract

Background: Colorectal carcinoma (CRC) is a primary cause of morbidity and mortality worldwide. Resistance to therapy contributes to poor patient prognosis. The aim of our study is to identify the key proteins and interaction networks implicated in CRC which may serve as possible therapeutic targets and help in overcoming therapy resistance.

Methods: The microarray dataset of 58 cases and 62 controls was used to identify Differentially Expressed Genes (DEGs).After constructing protein-protein interaction networks , Cytoscape analysis was done to identify the hub proteins. Based on sub graph centrality, between-ness and degree (⩾10), hub proteins were selected for further literature search and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results: A total of 85 up-regulated genes and 95 down-regulated genes of CRC patients were selected based on criteria of P>0.05 and fold change>2.0. The PPI analysis revealed *STAT3, HNRNPA2B1, RBM8A, RBM25, ATM, HIST1H2BK, SRSF5* and *HNRNPDL*as hub proteins. On the basis of criteria set for cytoscape analysis, *STAT3* and *HNRNPA2B1* were identified as key hub proteins. KEGG pathway analysis revealed vital role of *STAT3* in carcinogenesis.

Conclusion: In addition of *HNRNPA2B1* activation by *STAT3,* cross talk of *STAT3* with other oncogenic signaling pathways signifies its role in colorectal carcinogenesis. Our study highlights that*STAT3*may be a possible therapeutic target which may help in overcoming the dilemma of resistance to drug treatment in advanced cases.

Introduction

CRC denotes a primary cause of morbidity and mortality worldwide [1]. The World Health organization (WHO) data reports that out of 9.6 million cancer related deaths that occur globally, colorectal carcinoma (CRC) results in approximately 0.862 million deaths [2]. Bailey et al in 2015 documented that CRC risk will show an increase of about 90% in a decade [3,4]. Although CRC, if diagnosed at an early stage, is a curable disease it still remains the second most commonly reported cause of cancer related fatality [4]. The advancement of treatment modalities has achieved only a slight improvement in survival rate [5]. Mainstay of CRC treatment is surgery. If surgery fails to offer complete remission, target-based therapy, neoadjuvant radiotherapy and adjuvant chemotherapy is indicated. However, drug resistance remains one of the vital reasons for poor overall survival rate of CRC patients [5]. Patients experience treatment resistance and relapse of disease which may be attributed to plethora of molecular events defining complex pathogenesis of CRC [6,7]. Understanding of molecular events responsible for therapy resistance can open avenues for drug development and improved patient management [8].

It is imperative to explore possible molecular targets of colon cancer and to determine the molecular mechanisms associated with drug resistance. This will support the designing of novel strategies for successful treatment of patients with CRC [9,10]. Bioinformatics tools have gained popularity due to their use in collection, classification and analysis of biological datasets including the gene expression microarray datasets. The world has stepped towards precision medicine based on bioinformatics analysis for identifying the dynamic molecular events that determine disease pathogenesis [11]. Data mining of the available microarray datasets may act as a key source for understanding the molecular pathogenesis and for carrying out targeted experiments. Deeper understanding of genetic alterations in colorectal carcinoma and the functional consequences of these mutations can lead to improved therapeutic approach and better patient management.

Methods

Ethical consideration: The study has been carried out after approval from Ethics Review Committee of Ziauddin University (2861120SHPAT).The study was conducted in Multidisciplinary Laboratory Ziauddin University(Figure 1). In our study the microarray dataset was obtained from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/).

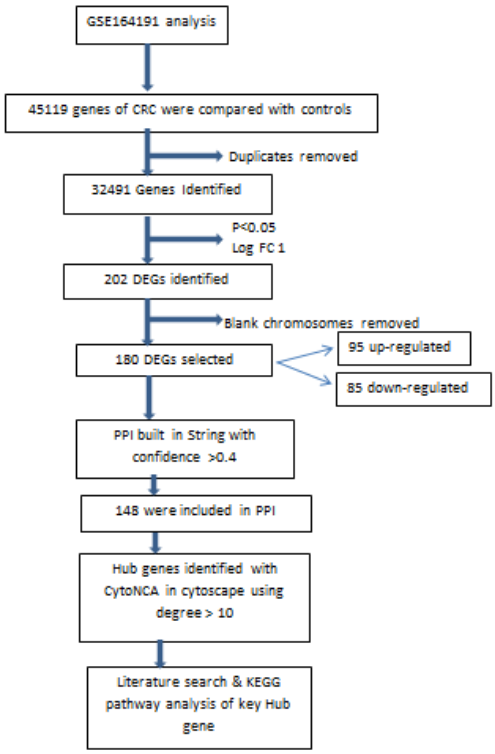

Figure 1: Selection criteria and workflow of the Study. After selection of DEGs, protein-protein interaction was studied using STRING which led to identification of HUB genes. Literature search and KEGG analysis of key Hub genes was carried out.

Identification of DEGs: We selected 121 samples, with 62 controls and 58 CRC case which were obtained from NCBI generated microarray dataset GSE164191. The identification of DEGs was done based upon p value to test the differential expression of the genes between the CRC and the control groups. The p value was calculated using the Student's t-test. The cut-off criteria were kept at the fold change>2.0 and a corrected $p < 0.05$.

PPI analysis: To perform the PPI analysis, online database Search Tool for the Retrieval of Interacting Genes was used. The parameter of interactions was set as confidence>0.4. To visualize and analyze the PPI network, Cytoscape software was used. The scattered proteins in cytoscape were removed from the final PPIs. The proteins, which worked like a hub in the network, were selected by CytoNCA on the basis of their interaction with other proteins. The selection was based upon degree centrality, between-ness centrality and sub graph centrality. The degree was set at ⩾10 for further selection of hub proteins.

Literature search: Manual literature search was performed to explore the role of the selected hub proteins in CRC.

KEGG Pathway analysis: The selected hub proteins were searched for associated pathways in human cancers using KEGG pathway analysis.

Results

DEGS: There were 95 up-regulated genes and 85 down-regulated genes among the total of 180 DEGs which were selected according to the defined criteria (Figure 2; Supplementary Dataset S1).

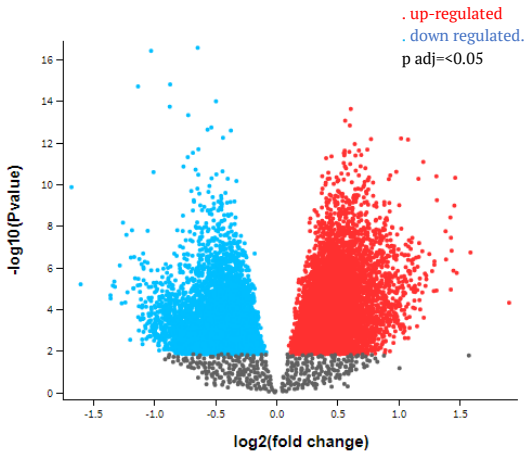

Figure 2: Volcano plot GSE 164191: Highlighted genes are significantly differentially expressed in blood-based analysis of colorectal cancer and healthy controls.

PPI network Analysis: After initial input of 180 selected DEGS , 148 DEGs with mean confidence score >0.4 were used to construct PPI (Figure 3).Identification of genes which were closely related with others was done using degree centrality, between-ness centrality and sub graph centrality ,and selected at degree ⩾10 (Table1). This analysis highlighted*STAT3, HNRNPA2B1, RBM8A, RBM25,ATM,HIST1H2BK, SRSF5*and *HNRNPDL* as key differentially expressed proteins.

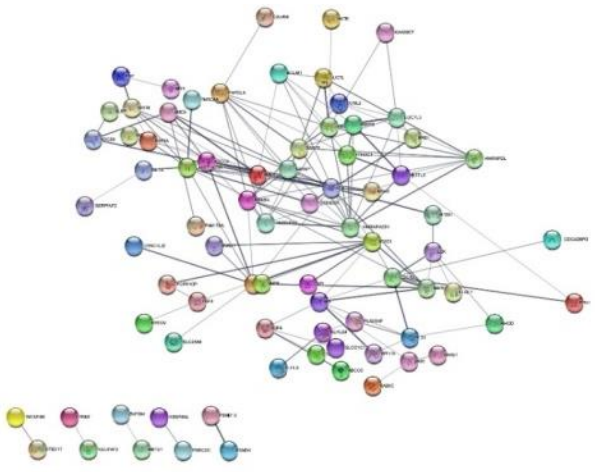

Figure 3: Protein-Protein Interaction network constructed with DEGs. The nodes represents proteins while the edges represent the protein-protein associations.

Selection of HUB gene: Manual literature search was done on the top hub genes, *STAT3* and *HNRNPA2B1* to identify their role in CRC. Based on the results of CytoNCA analysis and literature search, *STAT3* was chosen as hub protein. Literature showed a significant role of *STAT3* in colorectal carcinogenesis as well as in associated therapy resistance.

Degree	Sub graph	Betweenness
STAT3	HNRNPA2B1	STAT3
RBM25	SRSF5	RBM8A
HNRNPA2B1	RBM25	ATM
ATM	HNRNPDL	HIST1H2BK

Table 1: Hub proteins identified by Cytoscape analysis
STAT3 Signal Transducer and Activator of Transcription 3; HNRNPA2B1Heterogeneous nuclear ribonucleoprotein A2/B1; RBM RNA Binding Motif Protein (RBM25; RBM8A); SRSF5: Serine and Arginine Rich slicing Factor 5; ATM Ataxia-Telangiectasia and Mantle Cell Lymphoma;HIST1H2BK: Histone Cluster 1 H2B Family Member HNRNPDL: Heterogeneous Nuclear Ribonucleoprotein D Like

KEGG pathway analysis: KEGG pathway analysis of *STAT3* in human cancers (map 05200) revealed pathways associated with *STAT3* including cytokine receptor interactions, MAP kinase signaling pathway, *PI3K/AKT* signaling pathway and *JAK-STAT* pathway (Fig 4). KEGG pathway analysis did not reveal significant role of *HNRNPA2B1* in CRC.

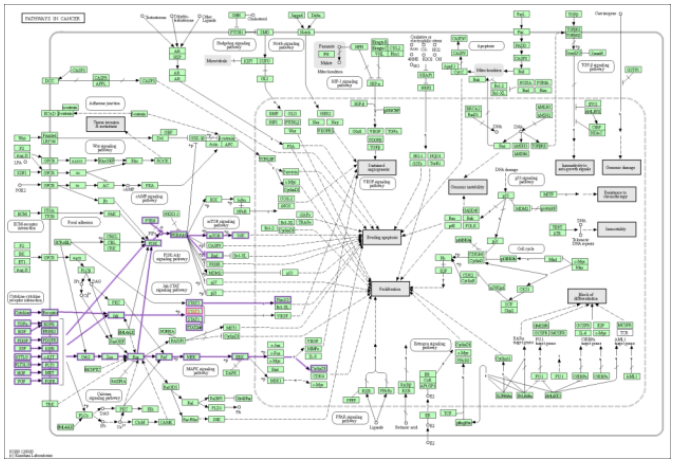

Figure 4: KEGG pathway analysis showing association of *STAT3* with several oncogenic pathways. (high resolution image is available in the HTML version of this manuscript).

Discussion

In our study we aimed to find out vital protein interaction networks in colorectal cancer. The comparison of dataset between normal controls and colorectal cancer patients revealed 180DEGs in which 95 were up regulated and 85 were down-regulated. The DEGS were selected for PPI construction which led to identification of two key proteins, heterogeneous nuclear ribonucleoprotein A2/B1 (*HNRNPA2B1*) and Signal transducer and activator of transcription 3 (*STAT3*) based on sub graphcentrality, degree and

betweenness.On exploring literature, we found association of these two proteins withcolorectal carcinogenesis. The primary role of *HNRNPA2B1* has been documented in pathogenesis of amyotrophic lateral sclerosis [12,13]. Studies have reported that*HNRNPA2B1*can promotingcolorectal tumour cell invasiveness [14]. It has been suggested that *HNRNPA2B1*facilitates tumor metastasis through extracellular regulated protein kinases (ERK) pathway [15]. Moreover, the expression and splicing of RAF kinase has been documented to be regulated by *HNRNPA2B1* [16]. Moreover, the heterogeneous nuclear ribonucleoproteins (HNRNPs) have been reported to be associated with *JAK STAT* signaling pathway with up regulation of *HNRNP*s via *STAT3* activation [17,18]. The *STAT3* gene is a member of the *STAT* protein family which regulates cellgrowth and proliferation [19]. Among the family of *STAT* proteins, *STAT3* has been reported to be overexpressed in about 70% cancer[20]. KEGG pathway analysis highlights the association of *STAT3* with human cancers.Activation of *STAT3* may be due to phosphorylation signals by cytokines like IL6, activated Janus kinases (*JAK*), activated epidermal growth factor receptor (*EGFR*) or by mitogen activated pathway (*MAP*) kinases. Activated *STAT3* can lead to increased transcription of target genes including cell-cycle regulator genes, proto-oncogenes, and anti-apoptotic genes. In this era of precision medicine,anti-EGFR drugs have gained popularity with *EGFR* being a possible therapeutic target in CRC patients especially in patients where surgery does not offer complete cure [21]. However, the development of resistance to EGFR-tyrosine kinase inhibitor drugs has narrowed their scope in CRC therapeutics [21]. Several kinases associated signalling pathways have beenimplicated as possible mediators of resistance to anti-EGFR targeted therapy [22,23] .Zulkifli et al documented upon the role of STAT3 signaling in providing tumour cells with an escape mechanism to inhibitory effects of anti-EGFR drugs [23]. Our search of KEGG pathways highlightsthe association of *STAT3* activation with EGFR which indicates that *STAT3* is an important protein contributing to stepwise accumulation of genetic events incolorectal Carcinogenesis. Hence anti EGR therapy resistance may be due to downstream activation of *STAT3*. Moreover, the role of *STAT3* in CRC chemotherapy resistance has been studied by *STAT3* inhibition which can sensitize colorectal cancer cells to 5-Florouracil therapy through down-regulating cyclinD1 [24]. This resistance may be attributed to *STAT3* activation through cytokine receptor activation. The phosphorylation of cytokine receptors is triggered by Janus kinase which leads to activation of cytokine receptor associated kinases namelyEGFR, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and receptor-associated kinases that activate *STAT3* [25]. *JAK2* and *STAT3* activation may play a significant role in promoting CRC metastasis [26]. Tsai et al. demonstrated that progression of colon cancer can be attributed to *JAK2* and *STAT3* activation by IL-6 [27]. Furthermore, the negative regulators of *STAT3* like suppressors of cytokine signaling are believed to be perturbed in malignancy [28]. Studies have reported that *STAT3* levels are higher in dedifferntiated colon cells and that there is a negative correlation between high levels of *STAT3* and prognosis of CRC [4].

Clinical trials are ongoing to explore efficacious target therapy based on inhibiting *STAT3*. However *STAT3* inhibitors have not been marketed as yet. Rational bioinformatics toolsalong with reliable assays are critical for attaining this aim. Because of the crosstalk of *STAT3* and other oncogenic signaling pathways drug repurposing approach for chemotherapy may lead to better cancer management. Furthermore, the development of *STAT3* inhibitors in combination with other therapies may help in formulating effective cancertreatment [29]. Our analysis highlights*STAT3* as the hub gene which is associated with CRC. Theassociation of *STAT3* with CRC related pathways highlights the need of further research on *STAT3* as a possible therapeutic target. This may help in overcoming the treatment failure associated with drug therapy such as anti-EGFR therapy resistance.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Sobia Hassan : conceptualization ,data acquisition, manuscript writing and editing, correspondence

Talat Mirza: designing the study, editing, revising and final manuscript approval

Ambrina Khatoon: data acquisition ,analysis and interpretation, manuscript writing

Uzma Bukhari: data analysis and interpretation, manuscript writing and editing

Fouzia Shaikh : manuscript writing and editing.

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