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Network and structure based inference of functional single nucleotide polymorphisms associated with the TGFβ1 gene and its role in colorectal cancer (CRC)

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ABSTRACT

System level analysis of the cellular signaling processes helps to understand the perturbations due to gene expression or through protein-protein interactions. In the case of colorectal cancer (CRC), a large proportion of patients display mutational inactivation of multiple pathways. Specifically, TGFβ-signaling is postulated to be one of the major contributors among them. The vast variety of studies to date considered the role of TGFβR1 and TGFβR2 receptors in CRC progression and relatively fewer studies targeted the Transforming growth factor-β1 (TGFβ1) protein. Therefore, this remains a poorly understood mechanism. TGFβ1 is the most abundant and universally expressed isoform, which is secreted into the extracellular matrix as a latent protein and is the important regulator of the cellular proliferation. Increased expression of TGFβ1 allows immunogenic cell lines to escape immunosurveillance and thus form tumors. Till date, no study has focused on the complete aspect of the TGFβ1 effects. Here, we investigated the consequences of 37 ns-SNPs in the TGFβ1 gene through computational tools and four ns-SNPs L28F (rs199946261), G46R (rs768250306), N69Y (rs763943753), and L83R (rs541829714) were identified that are highly likely to affect the structure, function, and activity of the TGFβ1 protein. Network motifs were also identified for the concerned pathway in the quest for functional annotation. In our study, we have performed a combination of network and structure-based analysis to elucidate the role of candidate genes for the TGFβ signaling pathway and to infer the mutational effect on the TGFβ1 gene and their relationship towards CRC progression.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths worldwide and it is expected to increase by an estimate 2.2 million new cases and 1.1 million cancer deaths by 2030 (Arnold et al., 2016). Globally, CRC is the second most common cancer in women (614,000 cases per year) and the third most common in men (746,000 cases per year) (Haggar and Boushey, 2009). The incidence rates are higher in developed countries (737,000 cases per year) than in developing countries (624,000 cases per year) (Haggar and Boushey, 2009). However, the mortality rate is higher in the developing ones and risk is relatively lower in women than in men (Tariq and Ghias, 2016; Hisamuddin and Yang, 2006). There are many factors such as chemical, environmental, lifestyle, etc., which are responsible for the CRC initiation and its metastasis (Haggar and Boushey, 2009; Grady and Markowitz, 2002). Also, increasing rate of CRC among the young population is due to several factors that include environmental (physical inactivity, obesity, smoking, red meat intake, and alcohol consumption) as well as genetic, such as mutation in candidate genes or due to the family history (Martin, 2003; Li and Martin, 2016; Markowitz and Bertagnolli, 2009).

A common cause of mutation in a gene is the faulty DNA repair mechanisms (Brown, 2002). DNA repair mechanisms play an important role in repairing the damages inflicted to the DNA and thereby regulate cancer progression (Markowitz and Bertagnolli, 2009; Gavande et al., 2016; Dietlein et al., 2014). However, it has been found from the studies that mismatch repair (MMR) mechanism act as a common etiologic factor in CRC (Peltomaki, 2001). Transforming growth factor-β1 (TGFβ1) decreases both the expression of Rad51 and Rad51-mediated

Abbreviations: CRC, colorectal cancer; TGFβ1, transforming growth factor-β1; MAPK, mitogen-activated protein kinases; PKC, protein kinase C; SNPs, single nucleotide polymorphisms; TFs, transcription factors; NCBI, National Center for Biotechnology Information; ns-SNPs, non-synonymous; PDB, protein data bank; SIMs, single input motifs; MD, molecular dynamics; RMSD, root mean square deviation; RMSF, root mean square fluctuation; Rg, radius of gyration; SASA, solubility accessible surface area

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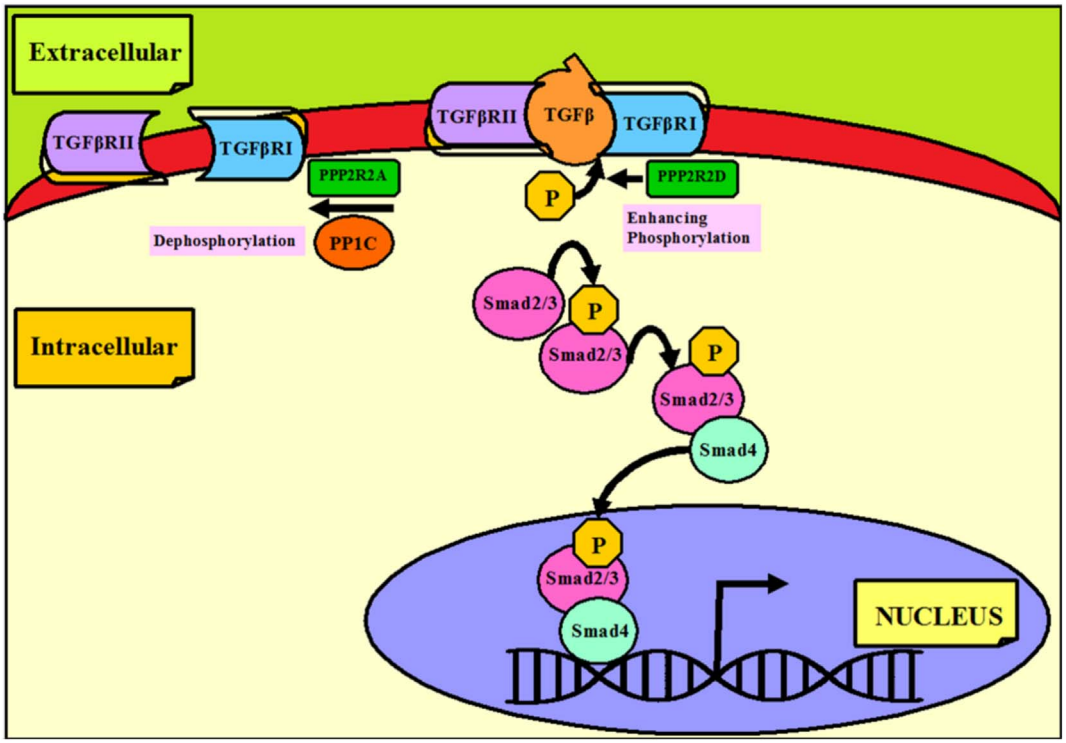

Fig. 1. Overview of TGFβ canonical signaling pathway mechanism. The signaling pathway initiated with binding of the TGFβ dimer (TGFβ1/TGFβ2/TGFβ3) to the Type 2 receptor (TGFβR2) at the cell surface, TGFβ dimer induces formation of the complex between Type 2 (TGFβR2) and Type 1 receptor (TGFβR1) both are serine and threonine kinases, once bound to TGFβ a Type 2 receptor phosphorylates and activates TGFβR1 receptor. Smad transcription factors transduce the signal downstream of TGFβ receptors, the activated Type 1 receptor phosphorylates a receptor-regulated Smad2/3 which then dimerizes with the Smad4. The Smad dimer translocates into the nucleus and with the DNA binding partner activates transcription of target genes.

DNA repair efficiency to promote DNA instability (Kanamoto et al., 2002). Elliott et al. reported that ~85% of all human cancers become resistant to the growth-inhibitory effects of TGFβ1 and the mechanism of its resistance are well defined in cancers like colorectal, pancreatic, prostate, and skin cancers (Elliott and Blobe, 2005; Singh et al., 2007; Cui et al., 1996; Park et al., 2000). In general, TGFβ1 induces and regulates apoptosis, which is frequently mediated by the Smad-dependent pathway (Yamamura et al., 2000). However, sometimes it activates Smad-independent pathways such as Ras/Raf mediated mitogen-activated protein kinases (MAPK) pathway (Fink et al., 2001; Hanafusa et al., 1999) that drives proliferation of human colon and prostate cancer cells as well (Park et al., 2000; Yan et al., 2001). The Wnt and TGFβ pathways cooperate to suppress colon (Takaku et al., 1998), and pancreatic (Cullingworth et al., 2002) tumorigenesis through several direct interactions between these pathways. The protein kinase C (PKC) pathway has also been shown to interact with the TGFβ pathway to regulate oncogenesis (Murray et al., 2002).

The TGFβ1 shows a pleiotropic effect i.e. possessing both tumor suppressor as well as tumor promoter activity (Villanueva et al., 1998; Grady et al., 1999). Various mechanisms for its tumor suppression and tumor activation have been proposed earlier. In the cells continuous cell TGFβ signaling down regulates hTERT (a catalytic component of human telomerase) and suppresses telomerase expression, thereby acting as a tumor suppressor. However, the induced expression has been noticed during carcinogenesis (Hahn, 2003). Also, several evidence supports the prominent role of TGFβ signaling in stimulating angiogenesis specifically through deletion of TGFβ1, TGFβR1, and TGFβR2 (Dickson et al., 1995; Oshima et al., 1996; Larsson et al., 2001). In terms of immunological effects of the TGFβ signaling, the increased expression of TGFβ1 allows the immunogenic cell lines to escape immunosurveillance and leads to the impulsive T-cell activation thus leading to the development of an autoimmune disease and form tumors (Torre-Amione et al., 1990). In CRC, TGFβ1 inhibits the proliferation of normal intestinal epithelial cells (Kurokowa et al., 1987) and regulates the proliferation and differentiation of normal colonic epithelium (Avery et al., 1993). TGFβ1 is the most abundant and ubiquitously expressed isoform of TGFβ (Xu and Pasche, 2007). In CRC, it escapes the tumor-suppressor effect and become resistant to growth inhibitory effect (Hoosein et al., 1989). Therefore, the study of TGFβ1 can be clinically beneficial for diagnosis of tumors. A probable method to abrogate the negative effects of TGFβ signaling is by blocking the effects of excessive TGFβ1 activity and to inhibit TGFβ1 binding to its receptor, which could be used as a therapy for treating advanced and metastatic disease. Therefore, targeting TGFβ pathway might be useful in attaining the putative biomarkers for CRC progression and also to target the other pathways downstream to design effective therapeutics for CRC.

To date, at least eight single-nucleotide polymorphisms (SNPs) have been shown to affect TGFβ1 expression (rs2317130, rs11466313, rs1800468, rs1800469, rs11466314, rs1800471, rs1800470, and rs11466316); some of these interfere with transcriptional regulation by affecting the binding of transcription factors (TFs), while others interfere with protein production (Cebinelli et al., 2016). Variation in TGFβ1, TGFβR1, and Smad3 seemed to influence survival after diagnosis of colon and rectal cancer (Slattery et al., 2010). In a study conducted by Slattery et al. both rs1800469 and rs4803455 were associated with colon cancer but not rectal cancer (Slattery et al., 2010).

Based upon the established and essential role of TGFβ1 and its other regulatory roles, we targeted its detailed studies in signature pathways along with site-specific structural mutational analysis (Schneikert and Behrens, 2007). Also, the normal proliferation of the precursor cells depends on permanent activation of this pathway. So, by keeping this in mind we have designed our study where we have performed the network and structure-based analysis to determine the role of TGFβ1 gene in the CRC progression. We hypothesize that to completely understand the disease it is essential to consider all possible factors. Therefore, we