Unraveling Biophysical Interactions of Radiation Pneumonitis in Non-Small-Cell Lung Cancer via Bayesian Network Analysis

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Appendix E: Mechanistic Biological Interpretation of the Results

Our study of genetic variants identified four SNPs: Rs1800469 (TGF-β1), Rs4803455 (TGF-β1), Rs1799983 (NOS3), and Rs6494633 (SMAD3) that were specifically prominent in our dataset. Rs1800469 (−509 C/T) is a SNP within the promoter of polymorphisms of the transforming growth factor beta1 (TGF-β1), a multifunctional growth factor involved in wound healing and radiation pneumonitis [1, 2]. All 49 SNPs in the SMAD3 gene were examined to identify eight haplotype blocks within the gene, and significant associations between haplotypes and overall survival were observed for haplotype block 5 of SMAD3 including Rs6494633 (intron G/A) [3]. SMAD3 is a TGF-β1-dependent nuclear transcription factor and has been recently described as a key down-stream mediator in fibrotic signaling pathways [4]. In addition to activating extracellular matrix synthesis, SMAD3 appears to mediate the inhibitory activity of TGF-β1 on matrix-degrading enzymes and increase expression of matrix metalloproteinase inhibitors [5]. Additionally, it has been proposed that SMAD3 is up-regulated through a TGF-β1-dependent pathway and acts as an important transcription factor during the fibrotic process following radiation injury [4]. Moreover, Flanders et al. proposed that SMAD3 plays a unique role in the cellular and tissue responses to wounding, and attenuation of SMAD3 signaling may improve wound healing in previously irradiated skin [6]. Hildebrandt et al. found that Rs1799983 in NOS3 genotype was associated with a 70% reduction in risk of pneumonitis, and their study also demonstrate a dose-related effect in inflammation-related SNPs [7]. Based on the pre-treatment BN data, all the above identified SNPs were primarily negatively associated with dosimetric parameters (Fig. 3*a*), whose intra-relationships minimally altered even during treatment (Fig. 5*a*). Additionally, during treatment, we have identified a negative association between smad3_Rs6494633 and Interferon-gamma (IFN_{Υ}), which impacted RP2 prediction via regulating TGF-β1 (Fig. 5*a*). Previous study identified a negative correlation between SMAD3 and IFN_{Υ}, where loss of SMAD3 upon genetic ablation caused increased IFN_{Υ} production by natural killers (NK) cells following

treatments with CD-16 and IL-12 [8]. Taken together, these findings support the relationships between SNPs and RP2 identifed in our BNs (Figs. 3*a* and 5*a*).

Damage caused by radiotherapy, such as RP, likely results from the interaction between pro- and anti-inflammatory cytokines, whether the inflammation occurs and to what severity depends on the inter-balance between these two categories of cytokines [9]. The role of IL-2 in the inflammatory process is complex, and the balance between the IL-2 pro- and anti-inflammatory effects is critical for an appropriate mounting and resolution of immune responses [10]. There is increasing evidence to suggest that IL-15 may play an important role in protective immune responses such as allograft rejection and the pathogenesis of autoimmune diseases, where mononuclear cell infiltration is its hallmark feature [11]. IFN_{Υ} is a cytokine that is produced primarily by activated CD4+ or CD8+ T cells and NK cells and is recognized as chief mediator of innate as well as adaptive immunity[12]. Among the biological activities of IFN_{Υ}, activation of macrophages is of key importance and IFN_{Υ} upregulates a variety of pro-inflammatory parameters such as IL-15[13]. Desai et al. have evaluated the cytokines secretion profile of human lung tumor cells, to compare their cytokine profile either before or after acute (6 Gy) and fractionated doses (3×2 Gy), and they observed that the secretion of certain cytokines was cell line-specific and that TGF-β1 was highly represented in irradiated conditioned medium rather than IFN_{Υ}. In addition, in all the cell lines studied, they showed that TGF-β1 increased markedly in a dose dependent manner[14, 15]. These findings corroborate the interactions found among dosimetric information, pre- and during cytokines shown in our BN (Fig. 5*a*). Moreover, our previous work demonstrated that patients who developed RP2 following radiotherapy had higher plasma TGF‑β1 levels compared with those prior to radiotherapy, unlike patients who did not develop RP2 [16], confirming the relatioship between SLP_TGF_β1 and RP2 observed in the BN at mid-treatment timepoint.

Furthermore, our study of genetic expression parameters identified two microRNAs (miR-223-3p and miR-191-5p) as upstream regulators of various cytokines. Both of them are among the top 25 microRNA up-regulated in non-muscle-invasive Bladder Cancer according to white blood cells (WBC)-

derived bio-specimen source[17]. Liang et al. showed that miR-223 overexpression down-regulates ATM expression and sensitizes U87 cells to radiation in vitro and in vivo[18]. In contrast, miR-223 is upregulated in T cell acute lymphocytic leukemia (T-ALL), Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma, and metastatic gastric cancer [19]. Mir-191 is one of the highly expressed and stable miRNA in human serum or saliva [20]. It has been found to be differentially expressed in lung cancer patients; however, it was shown to not alter cell cycle, proliferation or chemo-sensitivity of lung cancer cell lines[21, 22]. In the context of inflammatory response, miR-191 has been shown to be involved in human NK cell activation through IL-2 and IL-15 stimulations[23]; and this relationship is also captured in the BNs (Figs. 3*a* and 5*a*).

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