Abstract. Lung cancer was demonstrated to be the most lethal type of malignant tumor amongst humans in the global cancer statistics of 2012. As one of the primary treatments, radiotherapy has been reported to induce remission in, and even cure, patients with lung cancer. However, the side effects of radiotherapy may prove lethal in certain patients. In past decades, the transforming growth factor β1 (TGFB1) signaling pathway has been revealed to serve multiple functions in the control of lung cancer progression and the radiotherapy response. In mammals, this signaling pathway is initiated through activation of the TGFB1 receptor complex, which signals via cytoplasmic SMAD proteins or other downstream signaling pathways. Multiple studies have demonstrated that TGFB1 serves important functions in lung cancer radiotherapy. The present study summarized and reviewed recent progress in elucidating the function of the TGFB1 signaling pathway in predicting radiation pneumonitis, as well as current strategies for targeting the TGFB1 signaling pathway in lung cancer radiotherapy, which may provide potential targets for lung cancer therapy.

Key words: lung cancer, transforming growth factor β1, radiotherapy

1. Introduction

Lung cancer was the most lethal type of malignant tumor amongst humans in the global cancer statistics of 2012 (1). By morphological type, lung cancer is primarily divided into small cell lung cancer (SCLC) and non-SCLC (NSCLC) (2). Lung cancer cells are sensitive to radiotherapy, and thoracic radiotherapy can eradicate chemotherapy-resistant tumor cells (3,4). A retrospective study demonstrated that clinical outcomes following stereotactic body radiotherapy are equal or superior to surgery alone for overall survival in patients with stage I-II NSCLC (3). Two meta-analyses have indicated a statistically significant advantage with respect to overall survival associated with limited-disease SCLC following radiotherapy (5,6). However, radiation-induced pulmonary injury, local recurrence and distant metastasis have become substantial challenges to the successful management of lung cancer (7).

Due to its non-specificity, radiation kills rapidly mitosing cells, irrespective of whether they are cancerous or normal, resulting in damage to tissues that have an increased turnover rate, including skin, lung, bone marrow and gut epithelium (8). At present, the prognostic factors of lung cancer therapy are widely studied (9-12). A prior study revealed that the DiAcSpm/cutoff ratio (DASr) is significantly increased in NSCLC, and the DASr was revealed to be an independent negative prognostic indicator in patients with NSCLC who underwent complete resection (13). The overexpression of SRY-box 2 may serve as a positive prognostic factor in patients with stage III squamous cell lung cancer receiving adjuvant radiotherapy (14). Although understanding of the
molecular mechanisms underlying the development of normal lung tissue injury and tumor tissue response in radiotherapy has improved, transforming growth factor-β (TGFβ1) is the most important factor among the numerous cytokines and growth factors that contribute to the radiation-induced injury process (15). The present study reviewed the function of TGFβ1 in radiation-induced pulmonary injury and lung cancer response to radiotherapy, aiming to discuss the clinical use of this cytokine in lung cancer radiotherapy.

2. TGFβ1 signaling pathway

The TGFβ family is a group of pleiotropic growth factors that activate signal transduction cascades that serve important functions in carcinogenesis and tumor progression (16). TGFβ1, a ligand of the TGFβ signaling pathway, is present in numerous cell types. TGFβ1 is most highly concentrated in healing wounds, where it is released in large quantities from platelets (17). TGFβ1 then recruits monocytes and macrophages to the injury site (18), inhibits epithelial cell proliferation and enhances fibroblast maturation into post-mitotic fibrocytes that increase fibrous tissue production (19,20), thereby accelerating angiogenesis and extracellular matrix formation (21-23). In cancer cells, TGFβ1 serves a dual role. Initially, it functions as a tumor suppressor by inhibiting cell growth and inducing apoptosis (24). However, during the later stages of tumor development, TGFβ1 functions as a tumor promotor by inducing the epithelial-mesenchymal transition (EMT) in cancer cells, resulting in increased invasion and metastasis (24,25).

The signal transduction initiates with the TGFβ1 ligand binding to and activating TGFβ receptor II (TGFβRII), which then phosphorylates TGFβRI through activating its kinase. Phosphorylated TGFβRI phosphorylates the downstream elements of the signaling pathway via the regulatory SMADs (R-SMADs) SMAD2 and SMAD3, in the C-terminus of these R-SMADs. Phosphorylated SMAD2 may form a stable complex with SMAD3 and the co-SMAD SMAD4 and the resulting SMAD complex is then translocated into the nucleus (Fig. 1). This SMAD complex interacts with multiple transcription factors, which further increases their binding affinity and specificity for the target gene promoters, resulting in gene transcription alterations (24,25). Inhibitory SMAD may bind TGFβRII and attenuate signaling pathway activation (26). TGFβ1 may also signal via multiple alternative cascades, including the mitogen-activated protein kinase and protein kinase B signaling pathways (27,28), which may further mediate growth by inhibiting cell cycle progression and inducing apoptosis (27).

3. TGFβ1 serves as a predictor of radiation pneumonitis (RP) in lung cancer radiotherapy

Radiotherapy is the primary treatment for patients with inoperable, locally advanced lung cancer. This conventional treatment has been reported to induce remission in and cure patients with the disease (29). However, with conformal treatment planning, the side effects can prove lethal and radiotherapy may be ineffective due to the limitations imposed by normal tissue. This is particularly true in tumors that require increased doses of radiation or are located within or adjacent to sensitive organs (30). RP is considered the most serious, dose-limiting complication of radiotherapy (30). Growth factors are synthesized and secreted between a few h and days following irradiation, and may then continue for months. Cytokine plasma levels, including those of tumor necrosis factor, interleukin (IL)-1β, IL-6, and TGFβ1 serve a predictive function for RP, the influence of tumor-derived cytokine production on circulating plasma levels in irradiated patients with NSCLC was evaluated in a previous study (30). The expression of TGFβ1 increased in a dose-dependent manner following exposure to ionizing radiation (31). TGFβ1 was reported as one of the most important growth factors among the molecules expressed in tissues following radiation exposure, and is associated with the incidence of RP (32). Multiple studies have established the positive association between the severity of radiation-induced lung injury and TGFβ1 signaling activation (33,34). In a rat model of radiation-induced lung injury, fibrosis developed and was accompanied by increased expression of TGFβ1 and activation of the TGFβ1 signaling pathway (35). Furthermore, TGFβ1 activation by radiation has been demonstrated to occur at decreased doses and in an approximately dose-dependent manner between 10 cGy and 5 Gy (36). Serially measuring plasma TGFβ1 levels has been proposed to estimate the risk of RP and to assist in determining whether additional dose escalation may be safely applied in chemotherapy (37,38). Anscher et al (38) assessed whether TGFβ1 may also be used to predict the risk of developing pulmonary injury following radiotherapy. Anscher et al (39) performed a small clinical trial and determined that it was feasible to use TGFβ1 to guide radiation dose selection for patients with lung cancer. Furthermore, the expression of TGFβ1 in the sputum was reported to be a factor for predicting RP (40). TGFβ1 may be expressed in the sputum of patients with lung cancer, in whom macrophages are the main sources...
of TGFB1 expression (40). Patients with increased TGFB1 expression in the sputum following radiotherapy were associated with an increased incidence of RP compared with those with decreased TGFB1 expression (40). All the aforementioned approaches resulted in decreased expression and activation of TGFB1 and decreased activation of the SMAD-dependent TGFB1 signaling pathway (37-40). The observations of Anscher et al (38,39) suggest that plasma TGFB1 measurements may assist in identifying whether patients with lung cancer are candidates for radiation dose escalation or decrease. A previous study also revealed that the serum expression of TGFB1 increased significantly four weeks, and reached the highest recorded level eight weeks, following irradiation in a rat model (41). Furthermore, downregulating TGFB1 protected against radiation-induced lung injury in the rat model (41).

Meanwhile, circulating TGFB1 levels in lung cancer patients are elevated compared with people without cancer (42). The reason for the higher levels of TGFB1 appears to be associated with greater production and altered bioavailability of this cytokine (43). The mannose-6-phosphate/insulin-like growth factor receptor type II (M6P/IGF2R) was reported to serve a crucial function in these feedback process (44). Kong et al (45) demonstrated that if patients with lung cancer exhibited a loss of heterozygosity in M6P/IGF2R, they were significantly more likely to exhibit increased plasma TGFB1 levels and to develop radiation-induced lung injury compared with those with heterozygosity in M6P/IGF2R. Reactive oxygen species (ROS) are produced following radiation exposure (46), and have been revealed to activate latent TGFB1 expression (Fig. 2) (47). Mice genetically engineered to overexpress one of the isoforms of superoxide dismutase have been demonstrated to resist radiation-induced lung injury (48). Similarly, the administration of superoxide dismutase mimetic has been revealed to decrease the severity of lung injury in rats, in vivo (49).

However, the results of using TGFB1 levels to predict the risk of lung injury have been inconsistent among studies (50). A previous study reported that the return of plasma TGFB1 levels to the normal range following radiotherapy accurately predicted that patients would not develop RP (38). Another study failed to confirm that TGFB1 serves as a predictor of RP in lung cancer radiotherapy (51). Jaeger et al (37) did not confirm that increased levels of TGFB1 following the end of radiotherapy represented an independent additional risk factor for developing symptomatic RP. Further study revealed that CT/CC genotypes of the TGFB1 rs1982073:T869C gene were associated with a decreased risk of RP in patients with lung cancer treated with definitive radiotherapy compared with the other genotypes, and therefore may serve as reliable predictors of RP (52).

4. Predictive function of TGFB1 in lung cancer radiotherapy

Multiple studies have demonstrated that increased serum levels of TGFB1 following the initiation of radiation therapy were associated with radiation-induced lung injury, as aforementioned (32-34). Changes in circulating TGFB1 levels during radiation therapy may also be associated with the prognosis of patients with locally advanced NSCLC (53). In locally advanced NSCLC, decreased expression of TGFB1 during radiotherapy is associated with a more favorable prognosis (54).

Radiation may trigger the synthesis and secretion of TGFB1, and the activation of the intracellular TGFB signaling pathway, as evidenced by the phosphorylation of SMAD and transcriptional activation of a TGFB-responsive reporter gene in lung cancer cells (54). Radiation induced cells to increase their migration in response to recombinant TGFB1, and this was accompanied by the upregulation of TGFB1 expression (54). An increasing slope of the dose-response curve was associated with the C-509T single nucleotide polymorphism, suggesting that a polymorphism within the promoter region of the TGFB1 gene is associated with radiation sensitivity (55). Breast cancer cells pre-treated with the TGFB1 small molecule inhibitor LY364947 were radiosensitized, irrespective of sensitivity to TGFB1-induced growth inhibition (56). Consistent with increased clonogenic cell death, DNA damage was significantly decreased in breast cancer cells pretreated in vitro or in vivo with a TGFB1I kinase inhibitor (56). Furthermore, TGFB1-neutralizing antibodies increased radiation sensitivity and significantly delayed tumor growth in response to single and fractionated radiation exposure (56). These results suggest that inhibiting TGFB1 activity prior to radiation attenuates the DNA damage response and increases the radiosensitivity of breast cancer cells (56). However, whether TGFB1 may serve as an effective sensitizer in lung cancer radiotherapy has not yet been reported.

The signaling pathway induced by TGFB1 serves an important function in lung cancer cell growth and differentiation, and this pathway is associated with a significant predictive value in lung cancer radiotherapy. The present study further reviewed
reports on TGFβ1-associated microRNAs (miRNA/miR) and the mechanisms underlying EMT in lung cancer radiotherapy.

**TGFβ1 signaling pathway-associated miRNAs may influence lung cancer development and anti-metastatic treatment.** Multiple studies have demonstrated that miRNAs are associated with cancer development, invasion and metastasis (57,58), suggesting they may serve a function in lung cancer treatment. Previously, SMAD proteins of the TGFβ1 signaling pathway were revealed to regulate miRNA expression through transcriptional and post-transcriptional mechanisms (25). Animal studies with transgenic mouse models supported the conclusion that activating TGFβ1 increased the incidence of lung metastases from breast cancer (59), whereas blocking TGFβ1 activity decreased metastatic potential (60). An *in vitro* study demonstrated that the TGFβ1-miR-21-ROS signaling pathway in bystander cells was critical for bystander responses to manifest in NSCLC (Fig. 2) (61). Jiang *et al* (61) demonstrated that when NSCLC cells were irradiated with 5 Gy X-rays, the TGFβ1 signaling pathway was activated and the cells released certain signaling molecules, including TGFβ1, into the surrounding medium. These signaling molecules diffused to bystander cells and activated the TGFβ1-miR-21-ROS signaling pathway in these bystander cells (61). A prior study also revealed that TGFβ1-induced miR-183 silenced tumor-associated natural killer (NK) cells (Fig. 2) (62). However, the tumor cells evaded NK cell surveillance by generating an immunosuppressive environment through multiple factors, including TGFβ1 (63). NK cells and cytotoxic T lymphocytes exposed to TGFβ1 did not kill tumor cells in humans or mice, indicating that TGFβ1 exhibited an immunosuppressive function (64). Consequently, elevated serum TGFβ1 levels were associated with poorer prognosis, and observed in the metastatic stage of numerous types of cancer (65). *In vivo* depletion of TGFβ1 or blockade of the TGFβ1 signaling pathway may restore the NK cell-mediated anti-tumor response.

**TGFβ1 exposure results in EMT and the inhibition of growth arrest and apoptosis.** To improve understanding of the dual tumor-suppressive and tumor-promoting function of TGFβ1 in cancer cells, Gal *et al* (66) assessed the response of mammary epithelial cancer cells to short and long-term TGFβ1 exposure. Cell proliferation was arrested and apoptosis was induced following exposure to TGFβ1 for 2-5 days, whereas the surviving cells underwent EMT and became resistant to proliferation arrest and apoptosis (66). EMT was reversed using a pharmacological TGFβRI kinase inhibitor or by ceasing TGFβ1 exposure. In addition, the downregulation of TGFβ1-dependent signaling pathways in the transdifferentiated (TD) cells was reversed, and proliferation arrest and apoptosis induced, upon ceasing TGFβ1 exposure (66). This previous study concluded that suppressing the anti-proliferative TGFβ1 signaling pathway in TD cells may permit TGFβ1-dependent survival and EMT-enhancing signaling pathways to function to stimulate proliferation, survival and EMT at low, but sufficient, levels (66). Therefore, the TGFβ1 signaling pathway may be modulated to facilitate switching from tumor suppression to progression (66).

EMT is a determinant of radiotherapy sensitivity in NSCLC (Fig. 2) (67). Yasushi *et al* (67) evaluated the association between EMT and radiotherapy sensitivity using NSCLC cells induced to undergo EMT with TGFβ1. The expression of EMT markers in tumor specimens obtained from patients with lung cancer were immunohistochemically analyzed. EMT resulted in increased malignant potential and reduced sensitivity to radiation, and further altered certain cells into therapy-resistant sub-lines with TGFβ1 (67). In addition, increased evidence of EMT was detected in surgically resected specimens following radiotherapy compared with biopsy specimens prior to radiation treatment (66). The patients with EMT marker-positive tumors were associated with a decreased disease-free survival rate compared with those with EMT marker-negative tumors (67). It was concluded that the TGFβ1 signaling pathway was associated with insensitivity to radiotherapy due to EMT induction (67). Novel therapeutic combinations using TGFβ1 inhibitors or EMT-signaling inhibitors may be required to circumvent the resistance of lung cancer to radiotherapy. Furthermore, another study suggested that radiation induced a migratory or invasive phenotype in carcinoma, including lung cancer cells *in vitro*, through hyper-activation of the TGFβ1 signaling pathway (54).

However, a major challenge in developing accurate models of radiation-induced lung toxicity is inter-patient variation in inherent radiation sensitivity. The predictive value of TGFβ1 in lung cancer radiotherapy and the mechanism underlying how the activation of the TGFβ1 signaling pathway during radiotherapy contributes to metastasis, cancer stem cell formation and resistance to therapy via EMT induction, require further study.

### 5. Preclinical and clinical strategies for targeting the TGFβ1 signaling pathway in lung cancer radiotherapy

Radiation oncologists have focused on tolerating tissue injury by limiting the dose or volume of the normal tissue receiving radiation (68,69). Although these dose-volume associations have received attention to more precisely correlate dose, volume and the risk for normal tissue injury, certain disadvantages that may influence the risk of treatment-associated complications remain (70). TGFβ1 represents a target for molecular therapies designed to prevent or inhibit normal tissue injury following cancer radiotherapy (71). The evidence supporting the critical function of TGFβ1 in the development of normal tissue injury following cancer therapy was aforementioned. The present study further reviewed the strategies aimed at preventing normal tissue injury and sensitizing tumor cells by targeting the TGFβ1 signaling pathway.

Due to the vast number of potential targets in the TGFβ1 signaling pathway, multiple approaches have been adopted *in vitro* to prevent the binding of TGFβ1 to its receptor, including specific antibody-mediated neutralization of soluble TGFβ, or dominant-negative inhibition of TGFβRI and TGFβRII (71,72). The TGFβ-specific approaches inhibited radiation-dependent TGFβ1 secretion, the phosphorylation of SMAD and reporter gene activity, indicating that autocrine production of TGFβ and the subsequent activation of TGFβ1 induced these changes (54). After administering a single dose of 1.0 mg/kg anti-TGFβ1 antibody, delivered with the final fraction of the right hemithorax irradiation, to Fischer rats, Anscher *et al* (72) demonstrated that, compared with in the rats receiving radiation alone, inhibited fibrosis, TGFβ1 expression...
and TGFβ1-induced signaling were observed in the rats treated with a combination of radiation and an anti-TGFβ1 antibody. A TGFβRI inhibitor was also administered daily to a group of Sprague-Dawley rats with irradiated right lungs (72). The drug was administered from 7 days prior to irradiation until sacrifice, or for 3 weeks. There was significantly decreased fibrosis, TGFβ1 expression and chronic oxidative stress in the treated irradiated group as compared with in the untreated irradiated group (72).

A prior study indicated that the TGFβ1 signaling pathway may induce the overproduction of fibrous tissue in response to radiation through SMAD-independent signaling pathways, including the ABL proto-oncogene (ABL1) signaling pathway (71). The drug imatinib, which inhibits the ABL1 signaling pathway, was reported to inhibit the development of pulmonary fibrosis in NIH-3T3 and AKR-2B MEFs and in mice models (73). TGFβ1 was also revealed to signal via the Ras homolog family member D (RHO)/Rho associated coiled-coil containing protein kinase (ROCK) signaling pathway, independent of the SMAD signal transduction cascade (74,75). Targeting of the RHO/ROCK signaling pathway has been revealed to protect against radiation enteritis. In addition, administering superoxide dismutase mimics has been revealed to decrease the severity of lung injury in a Fisher-344 rat model (49). Radiation sensitized cells and further upregulated the expression of TGFβRI and TGFβRII, resulting in an increase in lung and pancreatic carcinoma cell migration via EMT (54). Strategies for inhibiting EMT and inhibitors of TGFβRI and TGFβRII should be taken into consideration for minimizing radiotherapy side-effects in clinical practice. Otherwise, deregulation of the TGFβ1 signaling pathway may be induced by oscillating miRNA levels, such as miR-183. Therefore, the use of therapeutic agents that facilitate the TGFβ1 signaling pathway and silence the expression of miR-183 may represent a promising strategy for activating the immune system in lung therapy (62). These types of approach result in decreased activation of TGFβ1 and the SMAD-dependent TGFβ1 signaling pathway, and inhibit ROS production.

6. Conclusions

To conclude, information regarding multiple aspects of the TGFβ1 signaling pathway in carcinogenesis has increased over the past years. TGFβ1 is considered a critical cytokine in the development of late normal tissue injury following lung cancer radiotherapy (37-39). Monitoring TGFβ1 in the plasma and screening for TGFβ1 polymorphisms may assist in identifying normal tissue injury risk in patients with lung cancer (38,52,55). Although the molecular mechanisms underlying the TGFβ1 signaling pathway and the SMAD effector complex have been previously established (24,25), upstream regulators of the TGFβ1 signaling pathway and the mechanisms by which they regulate the components of the pathway as the tumor develops require further study. Strategies targeting TGFβ1 have been demonstrated to decrease the severity of normal tissue injury in animal models (41,76). However, determining how such strategies may be effectively and safely applied in humans requires further clinical assessment.

Acknowledgements

The present study was supported by Scientific Program of Jiangsu Province (grant nos. BE2015631 and BK20151174), Changzhou Scientific Program (grant nos. CJ20160015 and QN201503); Changzhou Municipal Commission of Health and Family Planning (grant nos. QN201604 and ZD201602) and Changzhou High Level Medical Talents Training Project (2016CZLJ026).

References


