Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Interaction of TGF-b1 and radiation in fibroblasts as a model of wound healing in combination with postoperative and intraoperative radiotherapy

Autor: Lin Ma

Institut / Klinik: Klinik für Strahlentherapie und Radioonkologie

Doktorvater: Prof. Dr. F. Wenz

Radiation-induced fibrosis is a common long-term adverse effect of breast cancer patients after ionizing radiotherapy. Recently, Intraoperative Radiotherapy (IORT) giving a high single dose to the tumour bed during lumpectomy has shown relatively low levels of fibrosis in clinical trials. On the other hand, post-surgical fractionated radiotherapy of the whole breast results in increased complication levels when irradiation is started too early (less than 5 weeks) after surgery, i.e. before wound healing is complete. It may be hypothesized that interactions between irradiation and wound healing after surgery could play a role. The purpose of this project is to establish an in vitro model for studying mechanisms of interactions between wound healing and irradiation that are presumed relevant for the radiation-induced fibrogenic response.This would have direct implications for the use of radiosurgery and intraoperative radiotherapy.

Radiation induced premature differentiation of fibroblasts is considered as an important cellular mechanism contributing to radiation-induced fibrosis. Fibroblasts also play important roles in wound healing, once activated they migrate into the wound, proliferate and produce extracellular matrix (ECM) components to remodel ECM. The cytokine transforming growth factor (TGF)-b1 has been shown to control key steps in wound healing, such as fibroblast influx, formation of ECM, and cell proliferation. TGF-b1 is also an important cytokine in mediating radiation-induced fibrosis and has been called a “master switch” for fibrosis. This project used human primary skin fibroblast GS4 to establish in vitro models for wound healing (fibroblasts migration) and GS4 differentiation in fractionated irradiation after wound healing.

First, effects of TGF-b1 and radiation on fibroblast phenotype in vitro (proliferation, cell cycle distribution and differentiation) were tested. Fibroblast proliferation was not affected by TGF-b1 when tested by a proliferation assay using the vital dye AlamarBlue. A BrdU labelling new synthesizing cells (S phase cells) showed decreasing labelling index from ~18% to ~0% during cell growth to confluence. The presence of TGF-b1 showed a higher labelling index than the absence of TGF-b1 in the unirradiated cells (~8%) and irradiated cells (~5%), indicating a stimulating effect on GS4 cell proliferation (P=0.003). Furthermore, irradiation induced a clear G2 block in the cell cycle, and a prolonged G1 arrest (72h after irradiation) with a senescence-like cell phenotype. Conventional external beam radiotherapy after surgery was simulated by fractionated daily irradiation of cells grown to density arrest with and without TGF-b1 for 5 days, respectively. The results showed an enhancement of TGF-b1 on fibroblasts radiosensitivity (the slop of SF increased from 0.248 to 0.305; P=0.04). The ratio of fibroblasts in late (L) versus early (E) state of differentiation increased 1.85-fold (P=0.005) after irradiation in the absence of TGF-b1, while the increase was lost when TGF-b1 was present during proliferation to density arrest.

Second, the effect of radiation and TGF-b1, alone or in combination, to fibroblast migration was tested by an in vitro wound healing model to quantify fibroblast migration in a wounded cell layer (“scratch assay”). The wound were closed at 48 h (>90%) by fibroblast migration for non-treatment control, while, when TGF-b1 was present, the wound only can be closed by 50%-80% at 48 h depending on the concentration of TGF-b1 (P=0.03 when the concentration of TGF-b1 was 1 ng/ml), suggesting an

inhibitory effect of TGF-b1 on fibroblast migration. Furthermore, irradiation could slightly accelerate fibroblast migration (5%-8% wound closure more than unirradiated cells).

The interesting findings of TGF-b1 inhibiting fibroblast migration impelled us to study in deep the cell signalling pathways involved in this regulation. The inhibitor of TGF-b1 type I receptor, SB-431542 could strongly inverse TGF-b1-induced migration delay, but not totally (90% at 24h), indicating its main role in this regulation process and also other pathways existence. The Rho family of small guanosine triphosphatases (GTPases), including Rho, Rac, and Cdc42, has been found to play essential roles in cell migration. Rho-associated kinase (ROCK), a downstream effecter of Rho has also been put in centre poison for TGF-b1-regulated cell migration in recent studies. In order to investigate whether Rho proteins regulate the TGF-b1-induced migration delay in fibroblast, pharmacological inhibition of Rho, Rac1 and ROCK by their inhibitors (Pravastatin, Y27632 and NSC-23766, respectively) were tested in the scratch assay. Pravastatin showed no effect, while NSC-23766 and Y27632 showed partly reverse (45% and 84% at 24 h, respectively) to TGF-b1-induced migration delay, indicating their roles in the regulation of TGF-b1 to cell migration.

The results suggest that TGF-b1-induced inhibition of fibroblast migration in response to wounding is mediated by multiple pathways, including the TbRI/Smad pathway, the Rho/ROCK pathway and the Rac1 pathway. A hypothetical mechanism for the inhibition was via downstream activation of LIM kinase and inhibition of cofilin activity, resulting in increased stress fibre formation and reduced migration. However, phosphorylated LIMK and cofilin were not detected, indicating that LIMK/cofilin was not the major target for TGF-b1 induced inhibition of fibroblast migration. The finding that radiation has no adverse effect on or even stimulates migration whereas TGF-b1 is inhibitory suggests that the timing of TGF-b1 release after irradiation and during wound healing may be an important factor in successful wound healing after IORT.