

## **EFFECTS OF X-RAY ON THE SUPRAMOLECULAR ORGANIZATION OF TIGHT AND ADHERENS JUNCTIONS IN CULTURED MADIN-DARBY CANINE KIDNEY (MDCK) CELLS.**

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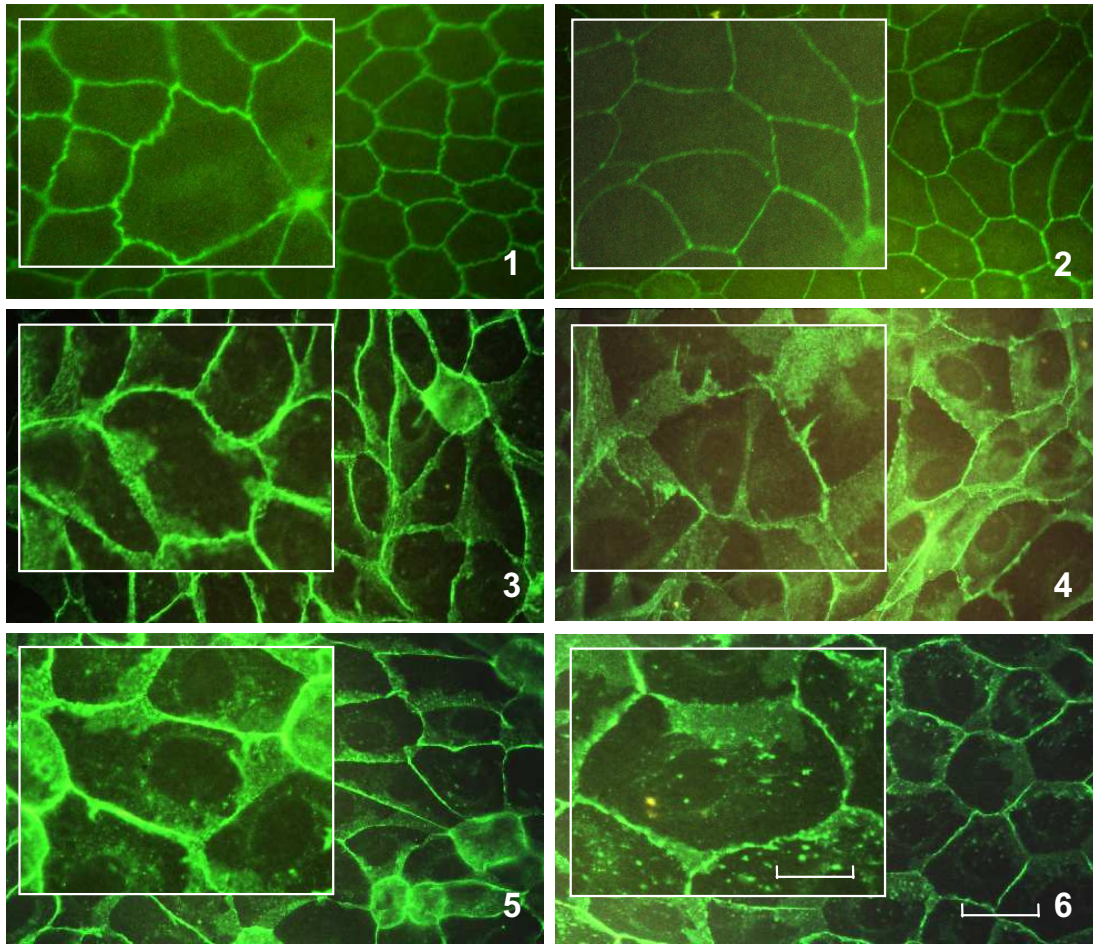
Tight and adherens junctions serve not only physical attachment of the neighboring cells, but regulate also paracellular transports of ions and other compounds, make a barrier for lateral shift of membrane proteins, and their role in different cellular signaling routes is also well known (1). The radiation response of junctional complex, their role in the development of radiation induced generalized symptoms (like inflammation, fibrosis, tumorous transformation, cell-death) are extensively investigated areas in radiobiology. The radiation-induced alteration of the paracellular permeability in different tissues (intestine, kidney, etc.) is considered to be the consequence of the structural alterations in the tight-, and adherens junction (2). In this work, we studied the subcellular and membrane localization of integral and associated proteins of tight- and adherens junction complexes (occludin, cadherins and  $\beta$ -catenin) by fluorescent microscopy (immunohistochemistry). Moreover, we also investigated the changes in the quantity of cadherins and  $\beta$ -catenin by Western blotting (3). For the examinations we used confluent cultures of MDCK cells maintained in standard conditions (3). The cells were fixed and permeabilized with methanol and stored at  $-20^{\circ}\text{C}$ . Fixed cell cultures were labeled with anti-occludin, anti-pan-cadherin and anti- $\beta$ -catenin antibodies (Sigma Company) and fluorescent secondary antibody. The results were investigated by Axioskope (Zeiss, Germany) 1, 4 and 6 hours after 0.5, 1, 4 and 8 Gy doses of X-ray irradiation. The dose rate was 0.317 Gy/min. In control MDCK cells, the tight junction protein occludin (Fig. 1), and adherens junction's pan-cadherin (Fig. 3) and  $\beta$ -catenin (Fig. 5) staining distributes mainly along the cell membrane appearing as a more or less continuous line, and shows characteristic honeycomb-like pattern at the junctional zone. We found changes in the staining characteristic of each antibodies even after 1-4 hours of irradiation by at least 1 Gy dose (Figs. 2, 4, 6). Continuous strands disappeared, the labeling crumbled and the intensity of the staining decreased which indicate dissociation of these proteins from junctional complexes of plasma membrane. Biochemical data obtained of western blots confirm the immunohistochemical results. We found, that irradiation caused a significant reduction in the amount of cadherins and  $\beta$ -catenin (Figs. 7 A, B).

Our presented results indicate that X-ray irradiation loosen the apical junctions of MDCK cells. According to the literature this means higher paracellular permeability, enhance tumorous transformation or apoptosis through signaling events involving adherens and tight junction associated proteins released into the cytosol.

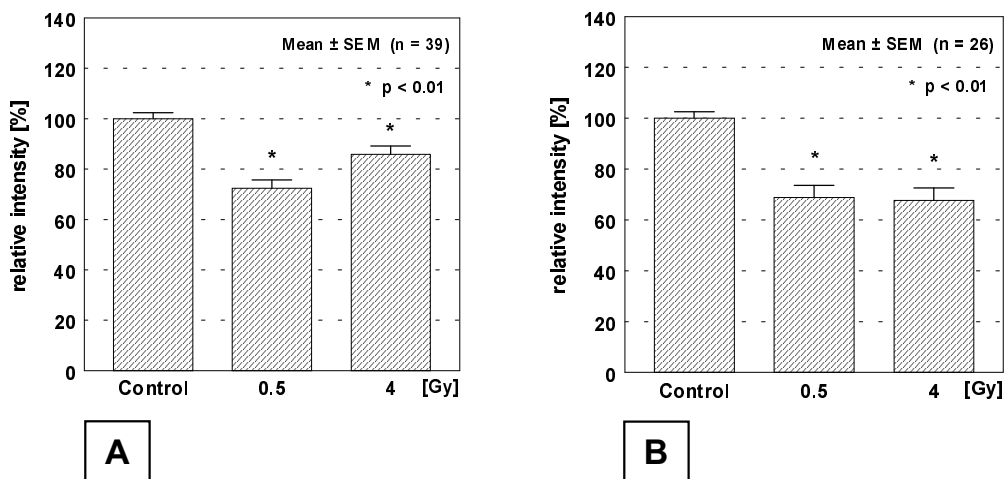
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### References

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**Figure 1-6.** Bars 10  $\mu\text{m}$ , inserts: 5  $\mu\text{m}$ . Binding characteristics of anti-occludin (Fig. 1: control, Fig. 2: irradiated by 4 Gy, 4 hour), pan-cadherin antibody (Fig. 3: control, Fig. 4: irradiated by 4 Gy, 4 hour) and anti- $\beta$ -catenin Fig. 5: control, Fig. 6: irradiated 4 Gy, 4 hour).



**Figure 7.** Changes in the level of cadherins (A) and  $\beta$ -catenin (B) in the control and irradiated (0.5 and 4 Gy 4 hrs after exposure) MDCK cells based on Western blot examination.