Response of organotypic slice cultures to ionizing radiation

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The aim of this work was to evaluate the response of liver and pancreas tissue to sparsely and densely ionizing radiation. For this, organotypic slice cultures (OSC) of liver (OLSC) and organotypic explant cultures (OEC) of pancreas (OPEC) were prepared from C3H wildtype (wt) mice and transgenic c-myc/TGF-α mice with an inducible liver tumor. OLSC from the transgenic c-myc/TGF-α mice were prepared from healthy (OHLSC) and neoplastic (ONLSC) parts of the liver. The pancreas from these animals was cultured as well (OTPEC). In order to evaluate a possible timedependence of the tissue response to ionizing radiation, OLSC and OPEC, OHLSC, ONLSC, and OTPEC were prepared at two different times of day: at the middle of the subjective day and at the middle of the subjective night.

Samples were cultured in a membrane-based culturing system with a liquid-air interface for several days. OLSC and OPEC from C3H wt-mice were irradiated with X-rays at doses of 2Gy, 5Gy, or 10Gy. OHLSC, ONLSC, OTPEC were irradiated with ^{12}C-ion extended Bragg peaks at the same doses. Mock-irradiated samples served as controls. All samples were fixed 1h and 24hrs post-irradiation, respectively, and immunohistochemically analyzed for markers of proliferation (Ki67), apoptosis (Caspase3), and DNA double-strand breaks (γH2Ax).

While the pancreas samples, unfortunately, did not produce any meaningful results with regard to the evaluated parameters, healthy liver tissue showed distinct day-night differences with regard to all three analyzed parameters: the proliferation rate was significantly increased at the middle of the subjective day compared to the middle of the subjective night. Contrariwise, the apoptosis rate and rate of DNA double-strand breaks was significantly increased at the middle of the subjective night. These day-night differences were not detected in ONLSC. Regardless of the radiation type and dose, irradiation of healthy liver tissue did not influence the evaluated parameters. In ONLSC, however, the rate of DNA double-strand breaks increased dose-dependently.

The effects of ionizing radiation on the circadian clockwork were further examined in tissue samples of transgenic Per2^{luc}-mice [1]. Per2^{luc}-mice express the enzyme luciferase under the control of the *Per2*-promoter, an important element of the circadian clockwork. Therefore, the analysis of these animals allowed to record the circadian rhythm of the molecular clockwork in liver and other tissues via realtime recordings of the luciferase-activity. As could be shown in OLSC and OEC from adrenal glands, ionizing radiation leads to a dose-dependent phase advance of the circadian clockwork.

The results of this project lead to the conclusion that ionizing radiation alter the circadian clockwork, but barely influence proliferation and apoptosis in healthy liver tissue.

References

[1] [Yoo2004]Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS „PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues“ Proc Natl Acad Sci U S A. 2004; 101(15): 5339–5346

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