Role of membrane Hsp70 in radiation sensitivity of tumor cells.

The major stress-inducible heat shock protein 70 (Hsp70) is frequently overexpressed in the cytosol and integrated in the plasma membrane of tumor cells via lipid anchorage. Following stress such as non-lethal irradiation Hsp70 synthesis is up-regulated. Intracellular located Hsp70 is known to exert cytoprotective properties, however, less is known about membrane (m)Hsp70. Herein, we investigate the role of mHsp70 in the sensitivity towards irradiation in tumor sublines that differ in their cytosolic and/or mHsp70 levels. The isogenic human colon carcinoma sublines CX(+) with stable high and CX(-) with stable low expression of mHsp70 were generated by fluorescence activated cell sorting, the mouse mammary carcinoma sublines 4 T1 (4 T1 ctrl) and Hsp70 knock-down (4 T1 Hsp70 KD) were produced using the CRISPR/Cas9 system, and the Hsp70 down-regulation in human lung carcinoma sublines H1339 ctrl/H1339 HSF-1 KD and EPLC-272H ctrl/EPLC-272H HSF-1 KD was achieved by small interfering (si)RNA against Heat shock factor 1 (HSF-1). Cytosolic and mHsp70 was quantified by Western blot analysis/ELISA and flow cytometry; double strand breaks (DSBs) and apoptosis were measured by flow cytometry using antibodies against γH2AX and real-time PCR (RT-PCR) using primers and antibodies directed against apoptosis.
related genes; and radiation sensitivity was determined using clonogenic cell surviving assays. CX(+/+) and CX(-/-) tumor cells exhibited similar cytosolic but differed significantly in their mHsp70 levels, 4 T1 ctrl/4 T1 Hsp70 KD cells showed significant differences in their cytosolic and mHsp70 levels and H1339 ctrl/H1339 HSF-1 KD and EPLC-272H ctrl/EPLC-272H HSF-1 KD lung carcinoma cell sublines had similar mHsp70 but significantly different cytosolic Hsp70 levels. ?H2AX was significantly up-regulated in irradiated CX(-) and 4 T1 Hsp70 KD with low basal mHsp70 levels, but not in their mHsp70 high expressing counterparts, irrespectively of their cytosolic Hsp70 content. After irradiation ?H2AX, Caspase 3/7 and Annexin V were up-regulated in the lung carcinoma sublines, but no significant differences were observed in H1339 ctrl/H1339 HSF-1 KD, and EPLC-272H ctrl/EPLC-272H HSF-1 KD that exhibit identical mHsp70 but different cytosolic Hsp70 levels. Clonogenic cell survival was significantly lower in CX(-) and 4 T1 Hsp70 KD cells with low mHsp70 expression, than in CX+ and 4 T1 ctrl cells, whereas no difference in clonogenic cell survival was observed in H1339 ctrl/H1339 HSF-1 KD and EPLC-272H ctrl/ EPLC-272H HSF-1 KD sublines with identical mHsp70 but different cytosolic Hsp70 levels. In summary, our results indicate that mHsp70 has an impact on radiation resistance.

Zeitschriftentitel / Abkürzung:
Radiat Oncol

Jahr: 2015
Band: 10
Seiten: 149
Sprache: eng

TUM Einrichtung:
Klinik und Poliklinik für RadioOnkologie und Strahlentherapie

Occurences:
- Einrichtungen > Fakultäten > Fakultät für Medizin > Kliniken und Institute > Klinik und Poliklinik für RadioOnkologie und Strahlentherapie > 2015

Entries: