

## 8 Summary

### Study of the effects of simulated microgravity on thyrocytes

The aim of the present study was to characterize the effects of simulated weightlessness on benign and malignant thyrocytes.

Three cell lines were used for the experiments: The follicular thyroid carcinoma cell line ML-1, the oxyphilic papillary thyroid carcinoma cell line ONCO-DG1 and the thyroid cell line HTU-5, derived from normal thyroid tissue. In this study, microgravity was simulated by a three-dimensional clinostat ('Random Positioning Machine'). The cells were cultured up to five days under conditions of 1 g and simulated 0 g. The study focus lies on the effects on morphology, the cytoskeleton, extracellular matrix proteins, and mechanisms of apoptosis.

We used phase contrast, fluorescence, confocal laser scanning and transmission electron microscopy, immunocytochemistry, DAPI-staining, TUNEL-staining, viability staining, as well as flow cytometry and western blot analysis including densitometry.

Formation of three-dimensional spheroids occurred within 12 h. Vimentin and vinculin as well as ECM proteins such as laminin, fibronectin, collagen type I and III, osteopontin, chondroitin sulphate were clearly enhanced after 24 h and 48 h of simulated microgravity compared to incubator or ground control cells.

Moreover, simulated microgravity induced apoptosis of thyrocytes. Throughout there was an increase in apoptotic cells among populations cultured under 0 g compared to control cells grown in an 1 g environment. Here, we demonstrate an increase in activated caspase 3, Fas, Bax, p53 and PARP and a decrease in bcl-2 for carcinoma cells. Other methods such as DNA-laddering, TUNEL staining, DAPI-staining proved apoptosis in all cell lines.

As early as 30 min, we could detect changes of the cytoskeleton concerning alterations of microtubules (tubulin) and intermediate filaments (vimentin, cytokeratin). Within 48 h the cytoskeleton was reorganized again.

In summary, for the first time this study showed the effects of simulated microgravity on human thyrocytes. ML-1, ONCO-DG 1 and HTU-5 cells formed multicellular spheroids. Moreover, simulated 0 g induced apoptosis, changed the cytoskeleton and increased the amount of extracellular matrix proteins. The three-dimensional clinostat represents an important tool for the formation of MCTS which can be used in tissue engineering for their production and culture. Multicellular spheroids are of great interest in oncology because they are very similar to *in vivo* tumors. In addition, this method helps to spare animal experiments. Furthermore, the clinostat as ground based facility offers a new possibility to perform experiments in the field of modern space medicine.