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Tierversuchen

Project

Development of an *in vitro* test system for cancerogenicity screening of chemicals with high throughput

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The process of tumour development is classically subdivided in three phases, namely initiation, promotion and progression. During the initiation step gene mutations can lead to the activation of proto-oncogenes or to the inactivation of tumour suppressor genes. In the course of the promotion stage initiated cells are stimulated to proliferate, so that preneoplastic lesions arise. During the progression phase further genetic alterations can result in the formation of a malignant tumour. Based on the above-mentioned multistep model of tumour development carcinogenic chemicals can be subdivided into two groups: 1) genotoxic compounds interact with DNA, thereby leading to irreversible genetic changes; 2) tumour promoters can enhance cell proliferation, particularly that of initiated cells, by a variety of different epigenetic mechanisms, whereby the compounds themselves do not induce irreversible genetic alterations in the cells.

Whether chemicals are carcinogenic can be determined by performing carcinogenicity studies in rodents. However, these studies are extremely time consuming (i. e. their carrying out and evaluation takes about three years to be completed) and costly (about one million Euros per study). If one takes into account the European legislation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), it is foreseeable that the carcinogenic potential of a number of chemicals will have to be tested in laboratory animals. One way of reducing the number of animals needed to prove the carcinogenicity of chemicals would be the development of an in vitro test system that effectively identifies compounds with carcinogenic properties.

The BALB/c-3T3 cell transformation test and the soft agar assay are two well established *in vitro* systems, which, if combined, would allow a simple quantitative analysis of the carcinogenic potential of chemicals. In the case of the BALB/c-3T3 cell transformation test a mouse fibroblast cell line is incubated with the test compound for a defined period of time. If the compound were carcinogenic, it will malignantly transform the cells. The malignantly transformed cells lose cell-cell contact inhibition, start piling up and form foci, whereby the number of foci formed is directly related to the transforming potential of the test compound. By making use of the soft agar assay one can definitely determine whether epithelial cells have been malignantly transformed. Non-transformed cells need direct contact to neighboring cells as well as to a firm underground. If this is not the case, the non-transformed cells will die off. In contrast, malignantly transformed cells can grow in soft agar (i.e. without a firm underground). Although the soft agar assay is the „gold standard“, when wanting to determine whether cells have been malignantly transformed, it has never been integrated before into an in vitro system to test the carcinogenic potential of chemicals.

In vivo most of the known carcinogenic chemicals must first be metabolically activated by drug metabolizing enzymes, in order to display their transforming capacity. Therefore, new *in vitro* systems to test the carcinogenic potential of chemicals should include by all means a metabolic activation step; by doing so they would better reflect the *in vivo* situation from a metabolic point of view.

In this project a metabolizing system will first be coupled to the BALB/c-3T3 cell transformation test. The subsequent combination of the BALB/c-3T3 cell transformation test with the soft agar assay will then allow for the first time to effectively test the carcinogenic potential of chemicals with a high throughput *in vitro*, thereby leading to a substantial reduction of the number of animals needed otherwise for this purpose.

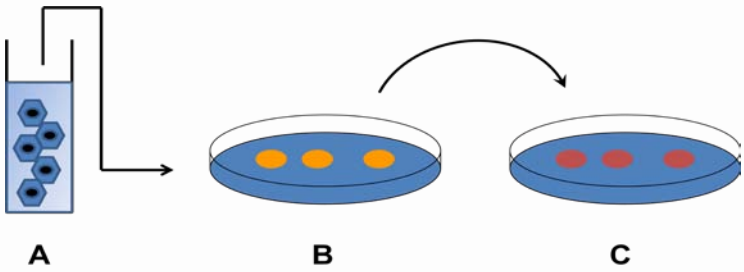

Fig. 1: Coupling of the metabolizing system to the BALB/c-3T3 cell transformation test: A) Incubation of the test substances with a liver homogenate; B) Cultivation of the BALB/c-3T3 cells with the supernatant of the incubation; C) Counting of the transformed cells

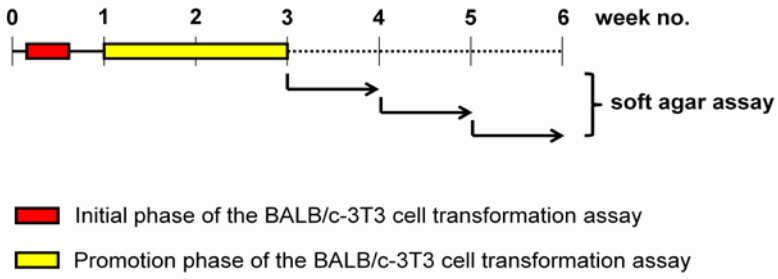

Fig. 2: Coupling of the BALB/c-3T3 cell transformation test to the soft agar assay

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