Division Mechanisms of Tumorigenesis (S0109)

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In the period of this report, the bilingual documentation of the first 20 years (1976-1996) of the DKFZ cooperation program with Israel has appeared [1]. It in e.g. the scientific and organizational development of cancer research in Israel and Germany in the first 100 years of the fight against cancer is acknowledged [2]. It comprises the foundation of the DKFZ (1964) under the leadership of the Deutsche Forschungsgemeinschaft as well as, independently but almost simultaneously, of corresponding research institutes in the former DDR [3]. On March 24, 1999, the scientific contributions to the generation of melanoma by the Japanese cancer researcher of outstanding merit, His Imperial Highness Prince Hitachi, upon proposal by the DKFZ, was honored by the Deutsche Krebsgesellschaft (DKG) as its first foreign honorary member. Upon this occasion a special issue of the Journal of Cancer Research and Clinical Oncology was issued comprising the scientific papers dedicated by reputed international cancer researchers to the new honorary member of DKG H.I.H. Prince Hitachi [4]. - The establishment of a new and modern, permanent exhibition on pharmacy at the Deutsches Museum, München, comprising important topics of biomedicine, was comprehensively advised. Under the motto “You are chemistry”, the exhibition displays i.a. aspects of cancer prevention and therapy and is intended to highlight the 100 year Anniversary of the world famous Museum in 2003 [5]. Publications (* = external co-author)


Main emphasis of publication of scientific results in the reporting period was laid onto the areas mechanisms of action (S0109-1/2) as well as environmental relevance of tumor promoters as risk factors of cancer (S0109-3). In the first area the hypothesis was investigated that activation of receptors of the diterpene ester (DTE) promoters in the proteome of target cells, i.e. of certain protein kinases C (PKC) of this multi enzyme family, leads to stimulation in the genome of these cells of biosynthesis of peptides or low molecular weight proteins regulating processes of cellular growth. In the second area, for quantitative estimation of the cancer risk introduced into the human environment by tumor promoters, based upon the general testing strategy developed previously (see Research Report 1997/98 and proceeding reports), special testing schedules were developed for defined doses of exposure, typical of certain utilizations of the suspicious environmental materials. In this way the tumor promoting risk of cancer may be estimated by quantification of the cancer risk factors contained in the actual exposure dose of an environmental material. Techniques were used to achieve this goal quickly and at low cost and the corresponding cancer risk may be expressed as scientifically sound so called “toxprognoses” for the experimental animal. The validity of toxprognoses of this kind and thus of the testing schedule employed, may be controlled by independent testing of the cancer risks of the suspicious materials in the two-stage model (initiation/promotion-protocol) on mouse skin. Valid toxprognoses of the cancer risk by tumor promoters (conditional cancerogens) in experimental animals achieved in this way, e.g. for purposes of regulatory toxicology, may be extrapolated to the risk of human beings in a similar way as it is long standing practice for the case of the classical solitary cancerogens. For areas of occupational, of alimentary or of iatrogenic cancer risks by tumor promoters in the human environment a few first toxprognoses were achieved in the reporting period (see also below and previous Ergebnisberichte or Research Reports).
Metabolism and Biochemical Mechanism of Action of Promoters of the Polyfunctional Diterpene Ester type (S0109-1,2)

E. Hecker, G. Krauter, R. Gminski, K. Schlatterer


As a plausible hypothesis on the molecular primary effects at the cellular level of tumor promoting diterpene esters (DTE) of the TPA-activity type the activation of certain protein kinases C (PKC) in the proteome of an initiated cell (hypothetical target cell of tumor promoters) may be considered. They may stimulate in the genome of the cells induction of certain peptides or of small molecular weight proteins regulating cellular growth, such as growth factors or transcription factors. An experimental approach to check this hypothesis would require demonstration of the appearance of new protein entities in murine epidermis before and after (in the I/P-protocol) tumor promoting singular TPA doses (e.g. Ergebnisbericht 1990/91, there page 87). In the period of this report by high resolution two-dimensional SDS polyacrylamid gel electrophoreses (2D-PAGE) in extracts of mouse skin before and after TPA application appearance of about seven new proteins was shown to be present in the molecular weight region up to 44kd for the first time [1]. Of these a protein with 10kd (p10) was isolated and its sequencing was started [2]. - On the tumor promoters of the okadaic acid-activity inhibiting the activity of protein phosphatases, independent investigations were reported by Japanese authors to show that okadaic acid stimulates the expression of the well-known cytokine tumor necrosis factor (TNFα) [3]. They hypothesize that a membrane attached form of TNFα may control cellular growth by an interkrine mechanism and indicate that also TPA induces expression of TNFα.

Publications (* = external co-author)

Tumorpromoters of the Diterpene Ester Type as Risk Factors of Cancer in Man (S0109-3)

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Research projects on the innovative utilization of species of spurge (Euphorbiaceae) aiming at renewable industrial raw materials are stimulated and indeed supported by the EC as well as by national granting agencies [e.g. by Fachagentur für Nachwachsende Rohstoffe (FNR), 18276 Gültow, Germany]. In the period of this report, after the wild type of E. lathyris (see previous research reports), as a further innovative utilization of species of spurge that of the wild type Euphorbia lagascae was analyzed regarding aspects of regulatory toxicology [1,2]. It is considered as another model case of human exposure by occupation to tumor promoters of the DTE-type. E. lagascae is known as a weed, growing preferentially in arid areas of Spain (e.g. around the city and in the region of Murcia). Its agricultural breeding at the experimental level aims at an innovative renewable source of vernolic acid (13-epoxy-9-octadeic-acid) or of corresponding seed oil lipids, and was part of a shared EC project. In the frame of this Dutch VOSFA project [Vegetable oils with specific fatty acids, (AIR-CT93-1817)], it was shown that the seed oil is a strong skin irritant. As main active principles, DTE of 12-deoxy-phorbol were demonstrated. In the two-stage-model on mouse skin [standardized initiation promotion (I/P) protocol 16] seed oil was shown to be a tumor promoter of medium degree activity (see Ergebnisbericht 1998/99).

Also all other parts of the plant of E. lagascae turned out to be skin irritant. In a subsequent EC supported “Concerted Action” led by ADAS Consulting Ltd. Wolverhampton, UK, as a partner of the activity, chemical analytics and toxicologic determinations of DTE promoters in the seed oil were developed [2-5]. Moreover, relatively simple measures of protection in handling and processing of the plants to be harvested, of the seed oil and of the waste materials (press cake, straw) were devised. As a conclusion it was recommended further for future activities to grow and select or to obtain by gene technological means special plant types of E. lagascae with low or no content at all of DTE-toxins, maintaining, however, simultaneously high yields of the product desired (vernolic acid) [6]. First trials of breeding, guided toxicologically, analogous to those suggested above for E. lagascae, in case of the wild type of E. lathyris (see above) were successful with regard to ecologically and economically innovative special types of the plant [6].

As a model case of alimentary exposure tumor promoters of the DTE type milk of cattle fed on fodder containing admixtures of skin irritant species of spurge is investigated. In the DKFZ cooperation project P513 (with the National Research Center in Cairo, Egypt) it was shown that e.g. in Egypt in fodder of goats frequently the herbaceous, skin irritant and hyperplasigenic Euphorbia species E. peplus, E. helioscopia and E. nubica occur. The transfer into the milk of mother goats of DTE promoters contained in E. peplus admixed to the fodder was already demonstrated (see Research Report 1998/99). In developing this model of alimentary exposure, in a further protocol mother goats were held on fodder contaminated by E. helioscopia and E. nubica, respectively [7]. Feeding of the...
contaminated fodder for four weeks (30 g/day/goats), resulted in definite appearances of intoxication in the mother goats, analogous to that seen in case of *E. peplus* contaminated fodder. Also, by the milk of the mother goats their suckling kids were intoxicated, causing death of the kid in singular cases. By use of microchemical techniques (HPLC) it was found [8], that the plant materials fed in the present protocol contained skin irritant promoters of the ingenane type (*E. helioscopia*) or skin irritant promoters of the tigliane type (*E. nubica*). In a further step the contaminated goat milk will be investigated to see determine the amount of DTE promoters originating from the contaminating plants. In a testing schedule to be developed (following our general testing strategy, see above) for such milk, the risk of carcinoma of the esophagus associated with its consumption may be estimated by an appropriate toxprognosis to be obtained.

As model case for drug associated exposure to tumor promoters of the DTE type, certain phytomedical preparations from *Euphorbiaceae* (spurge) and *Thymelaeaceae* (mezereon) may be used. In the period of the report, for commercial homeopathic mother tinctures of the preparations from *Euphorbiaceae* (spurge) and(50,708),(514,894)

![Fig. 1: Euphorbia lagascae spreng. a) entire plant, b) leave of the main stem, c) small leave of the flower, d) flower e) capsule with small leaves, f) ripe capsule, g) seed with karunkula, view from in front and from the side](image-url)