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Active Cell Death (ACD):
Concepts, Subtypes and Quantitative Detection in vivo

Abstract

The occurrence of cell death as a physiologic event in multicellular organisms has been known since more than 150 years. In 1972 the term apoptosis was introduced on morphological grounds. Accumulating evidence suggests that cells seem to use different pathways for active self-destruction as reflected by different morphology: condensation prominent, type I or apoptosis; autophagy prominent, type II; etc. Morphologically different types of ACD were found to be associated with different biochemical and molecular events eventually leading to cell collapse. Thus, induction of transglutaminase and cytoskeleton breakdown appears to occur during type I (e.g. hepatocellular apoptosis; HT29/HI1) but not during type II ACD (e.g. autophagic, lysosomal ACD of mammary tumor cells (MCF-7)). Furthermore, discrimination between active cell death and necrosis may not always be unequivocal. Thus, DNA fragmentation as detected by TUNEL assay and DNA ladder has also been found in necrotic cells. Currently no simple biochemical or molecular marker for detection of cell death subtypes is available. Therefore, for unequivocal identification of the various types of cell death morphological (electron- and light-microscopy), biochemical and functional criteria should be used in combination. During tumor development in various organs of animals and humans not only rates of cell proliferation (α), but also rates of cell death (β) may increase with increasing malignancy. Morphological and functional criteria (anti-promotion, withdrawal of survival factors, net growth rate α-β) indicate that ACD is a major determinant for the development and growth of tumors.

Concepts on Cell Death

The occurrence of cell death under a variety of physiological and pathological conditions in multicellular organisms has been documented manyfold during the past 150 years (Vogt 1842 for review: Majno and Joris, 1995, Clarke and Clarke 1995). Virchow (1871) described the diversity of cell death as 'necrosis' and 'necrobiosis'. Subsequently, cell death was reported to occur during the development of mammals

and metamorphosis of invertebrates and lower vertebrates, in hormone-dependent tissues such as prostate and mammary gland after removal of trophic hormones by gonadectomy, after treatment with glucocorticoids in lymphocytes and leukemic cells ('cytolysis'), and after various kinds of damage by chemicals, injury, radiation, hypoxia etc. In developmental biology cell death early was recognized as a 'programmed' event (Glücksmann 1951, Saunders 1966, Lockshin and Williams 1964, 1965). On the other hand, in toxicology and pathology cell death traditionally was considered as a passive, degenerative phenomenon occuring after severe damage of tissues. It was not before the early 1970ies when Farber et al. — based upon a characteristic morphology of cell death and its requirement for protein synthesis — suggested the occurrence of an 'active' or 'suicide' type of cell death in liver, intestine, and other organs after treatment with cytotoxic anti-cancer drugs (Farber et al. 1972). Nevertheless, the widespread occurrence and biological relevance of active cell death was only recognized by Kerr, Wyllie and Currie and in 1972 they proposed a classification of cell death into two broad categories. According to this proposal the term 'apoptosis' was coined to describe a category of cell death 'which appears to play a complementary but opposite role to mitosis in the regulation of animal cell populations. Its morphological features suggest that it is an active, inherently programmed phenomenon which can be initiated or inhibited by a variety of environmental stimuli, both physiological and pathological' (Kerr et al. 1972). The term 'necrosis' which usually was used for all types of cell death was re-defined and restricted to events caused by environmental perturbation, 'which must be violent and lead to rapid incapacitation of major functions and to collaps of internal homeostasis' (Kerr et al. 1972). This proposal initiated a controversial discussion on cell death in the scientific community but eventually helped to elucidate that active cell death is as important as cell proliferation for normal development and regulation of cell homeostasis in organisms.

In the last decade, a significant progress has been achieved in understanding the control of apoptosis by survival and death factors as well as the intracellular events eventually associated with cell suicide (for review: Tenniswood et al. 1992, Dexter et al. 1994, Schulte-Hermann et al. 1995, Peter et al. 1996, Cohen 1996, Kroemer et al. 1997, Lockshin 1997). Interest in cancer research was triggered when it was recognized that tumors may exhibit high rates of active cell death (Kerr et al. 1972, Gullino 1980, Sarraf and Bowen 1986) and that tumor promoters may selectively increase survival of preneoplastic cells (Bursch et al. 1984). Subsequently, blockade of apoptotic elimination of B-cells consequently to overexpression of the oncogene bcl-2 was found to be involved in the pathogenesis of Burkitt lymphoma, showing that prevention of apoptosis is not a phenomenon specifically for liver tumor promotion (Vaux et al. 1988). Unfortunately, the knowledge about apoptosis to date still is scarce and sometimes misconceptions are apparent which may obscure the clear recognition of phenomena and access to underlying causes. Thus, apoptosis often is equated with 'programmed' or 'active' cell death (ACD). However, accumulating evidence suggests that ACD is not confined to apoptosis as originally defined (see below; Schweichel and Merker 1973, Clarke 1990, Tenniswood et al. 1992, Schwartz et al. 1993, Zakeri et al. 1995, Bursch et al. 1996).

Types of Active Cell Death

Apoptosis originally was defined on the basis of a specific pattern of morphological changes in the dying cell (Kerr et al. 1972): condensation of cytoplasm, in solid tissues separation from neighbouring cells, condensation of chromatin at the nuclear membrane to sharply delineated masses and cell fragmentation into apoptotic bodies. In highly condensed dead cells or cell fragments organelles are still intact as shown by electron microscopy; cellular membranes are well preserved and consequently, cell contents are not liberated. In vivo, apoptotic bodies are rapidly phagocytosed and degraded by neighbouring cells. An important feature of apoptosis is that lysosomes (autophagy) do not play a distinct role in early stages but are involved later in the heterophagic degradation of apoptotic bodies (Kerr et al. 1972, Wyllie et al. 1980, Bursch et al. 1985). However, Schweichel and Merker (1973) and Clarke (1990) noted that active cell death may exhibit morphological features different from apoptosis. These authors described 3 morphologically distinct types of cell death in the developing embryo: type I is most likely identical to apoptosis. Type II is characterized by a prominent formation of autophagic vacuoles ('autophagic cell death'). In vivo, cell residues undergoing type II cell death, like those of apoptosis (type I) are finally phagocytosed by neighbouring cells (Schweichel and Merker, 1973). Type III is described as occurring through disintegration of cells into fragments without involvement of the lysosomal system and without marked condensation (Clarke 1990).

We have studied the occurrence of different types of cell death in human mammary carcinoma cells (MCF-7) and in the liver in vivo as well as in vitro. As to mammary carcinoma cells, previous studies on MCF-7 cells revealed an inhibitory effect of antiestrogens on DNA synthesis and enhanced cell death (Bardon et al. 1987, Jordan and Murphy 1990, Wärri et al. 1993). We have confirmed and extended these results using tamoxifen, 4-hydroxy-tamoxifen and ICI 164.384 (Bursch et al. 1996). Tamoxifen at high doses (10^{-5} M) causes lysis (necrosis) of almost all cells within 24 hours, which cannot be prevented by estradiol. The cytotoxic action of tamoxifen may result from perturbations in membrane fluidity (Wiseman, 1994), formation of reactive oxygen species (Tuner et al. 1991), DNA damage by DNA adducts or chromosomal aberrations, which have been found to occur in kidney and liver (Han and Liehr, 1992, Sargent et al. 1994). On the other hand, lower concentrations of tamoxifen (10^{-6} M and below) or ICI 164.384 induce a gradual appearance of cell death starting to occur 3 days after treatment. This type of cell death is considered to be a receptor-mediated, active cell suicide because of its inhibition by estradiol ('mitogen rescue') even six days after anti-estrogen treatment. In view of the screening for death factors such as antiestrogens it is worth to note that our studies with MCF-7 cells suggest that the anti-survival and anti-proliferative activity of antiestrogens can be regarded, at least to some extent, as independent, distinct pharmacological properties: 1. the anti-survival effect of tamoxifen and ICI 164.384 becomes manifest several days later than their anti-proliferative effect; 2. the lowest tamoxifen and ICI 164.384 concentration tested (10^{-8} M) exerts an anti-proliferative, but no anti-survival effect; 3. estradiol reversed the anti-survival effect of ICI 164.384, but not its

anti-proliferative effect; 4. tamoxifen and ICI 164.384 differed about 10-fold in anti-proliferative potency, but their anti-survival effect was equal.

Tamoxifen Induced ACD of MCF-7 Cells: Nuclear Changes

Electron microscopical studies revealed that the nuclei of MCF-7 cells undergoing ACD showed one of two distinct changes: a) apoptosis-like condensation and fragmentation of chromatin to crescent masses abutting to the nuclear envelope. b) condensation of the chromatin to a single, pyknotic mass in the center of the nucleus, detached from the nuclear envelope. Light microscopic evaluation revealed the predominance of the pyknotic type of cell death. Biochemically, activation of a non-lysosomal endonuclease that degrades DNA into (oligo)nucleosomes yielding a characteristic 'DNA-ladder' after gel electrophoresis is often regarded as halmark of apoptosis (for review: Arends et al. 1990, Bursch et al. 1992a, b, Schulte-Hermann 1995). However, apoptosis may not always be associated with DNA fragmentation into (oligo)nucleosomes, as exemplified by TGF-ß1-induced apoptosis in primary hepatocyte cultures (Oberhammer et al. 1993). Recent concepts suggest a sequential DNA degradation into high and low molecular weight fragments, the degree of which seems to depend on cell type and stimulus under study (Walker et al. 1995). As outlined above, most of the dying MCF-7 cells after Tamoxifen morphologically exhibit nuclear pyknosis which clearly differs from 'classical' apoptotic nuclei. However, by pulsed field and conventional gel electropheresis DNA fragmentation into large fragments (50 Kb) and further into oligonucleosomal fragments could be demonstrated (Bursch et al. 1996). Apparently apoptotic and pyknotic types of chromatin condensation may share DNA fragmentation into high molecular fragments. Studies on the biochemistry of DNA degradation underlying the different morphological features of nuclear changes are in progress.

Tamoxifen Induced ACD of MCF-7 Cells: Cytoplasmic Changes

By electron microscopy we noted early formation of autophagic vacuoles (AV) and a gradual loss of cytoplasmic organisation during active cell death. The number of cells with a normal looking nucleus but signs of extensive autophagy exceeded the number of those cells exhibiting a pyknotic or apoptotic nucleus, i.e. irreversible signs of cell death. The importance of autophagic vacuoles in the preparation of cells for death is supported by the inhibition of both types of nuclear alterations by 3-methyladenine (3-MA; Bursch et al. 1996). 3-MA has previously been characterized as a specific inhibitor of formation of autophagic vacuoles in liver cells (Seglen et al. 1982). In view of functional criteria for the differentiation between subtypes of ACD it is of interest to mention preliminary data suggesting that 3-MA does not inhibit TGF-ß1 induced 'classical' apoptosis of hepatocytes (W. Parzefall, personal communication).

Interestingly, in Tamoxifen treated MCF-7 cells structures required for protein synthesis such as polyribosomes, ER, and Golgi disappear completely, whereas a few clusters of intact mitochondria persist in close vicinity with AVs and the nuclear envelope. Remarkably, most of the dead cells remain attached to the substrate until 8-10 days after treatment (Bursch et al. 1996). In these cells the integrity of cytokeratin,

F- and G-actin appear well preserved. This was demonstrated in individual cells by parallel staining with Hoechst fluorescent dye (H 33258), which exhibits the nuclear structure, and with antibodies directed against G-actin and cytokeratin; F-actin was detected by FITC-phalloidin. Cytokeratin of cells detached from the substrate, however, shows signs of degradation into smaller fragments as detected by Western blot analysis. The protein cross-linking enzyme transglutaminase, which is activated in apoptotic hepatocytes (Bursch et al. 1992), apparently is not involved in tamoxifen induced ACD of MCF-7 cells. Currently, we investigate the mechanisms of cytoplasmic degradation in MCF-7 cells in more detail.

In conclusion, the features of antiestrogen-induced ACD in MCF-7 cultures are clearly different from those of TGF-ß1 induced apoptosis (electron microscopically proven) in the liver in vivo and in primary hepatocytes cultures previously described elsewhere (Bursch et al. 1985, Oberhammer et al, 1991; for review: Schulte-Hermann et al 1995). Rather, antiestrogen induced death of MCF-7 cells resembles that of type II or autophagic cell death (Schweichel and Merker, 1973, Zakeri et al. 1995). The most prominent morphological features of both types of cell death are schematically depicted in figure 1. Type II cell death has been described to occur in vivo under a variety of biological conditions (Schweichel and Merker 1973, Clarke 1990, Zakeri et al. 1995). It therefore appears that the autophagic mode of cell death is not peculiar to MCF-7 cells but is an important mechanism in tissue homeostasis of wide-spread importance.

No specific biochemical pattern has so far been attributed to type III cell death. Schwartz et al. have identified a type of cell death in insect tissues characterized by strong expression of the polyubiquitin gene and of the multicatalytic proteinase (proteasome) (Schwartz et al. 1993, Jones et al. 1995). Ubiquitin binds to cellular proteins to label them for proteolytic degradation by the proteasome protease. So far, enhanced expression of ubiquitin was not observed in models of apoptosis (Schwartz et al. 1993). Likewise, in our studies MCF-7 cells treated with tamoxifen showed no induction of ubiquitin expression above controls.

In conclusion, the different morphology and biochemistry of dying cells most probably reflects differences in cellular signalling and mechanisms. However, as recently pointed out by Zakeri et al. (1995) type I cell death (apoptosis) and type II may not be strictly separated or mutually exclusive phenomena. As shown in our MCF-7 cell culture studies, a subfraction of dying cells shows autophagic cell death with an apoptotic nuclear morphology. Therefore, in a given cell type several pathways leading to active death may coexist.

Active Cell Death versus Necrosis

An apparent misconception on apoptosis and ACD is that its discrimination from necrosis is unequivocal and, in principle, always possible. First, toxic injury may cause necrosis or active cell death depending on the extent of damage to the membrane, cytoskeleton or vital functions such as ATP-synthesis etc. As exemplified by the effect of different doses of tamoxifen on MCF 7 cells (see above) there may exist a threshold of injury beyond which necrosis occurs in a given cell. Single hepatotoxic

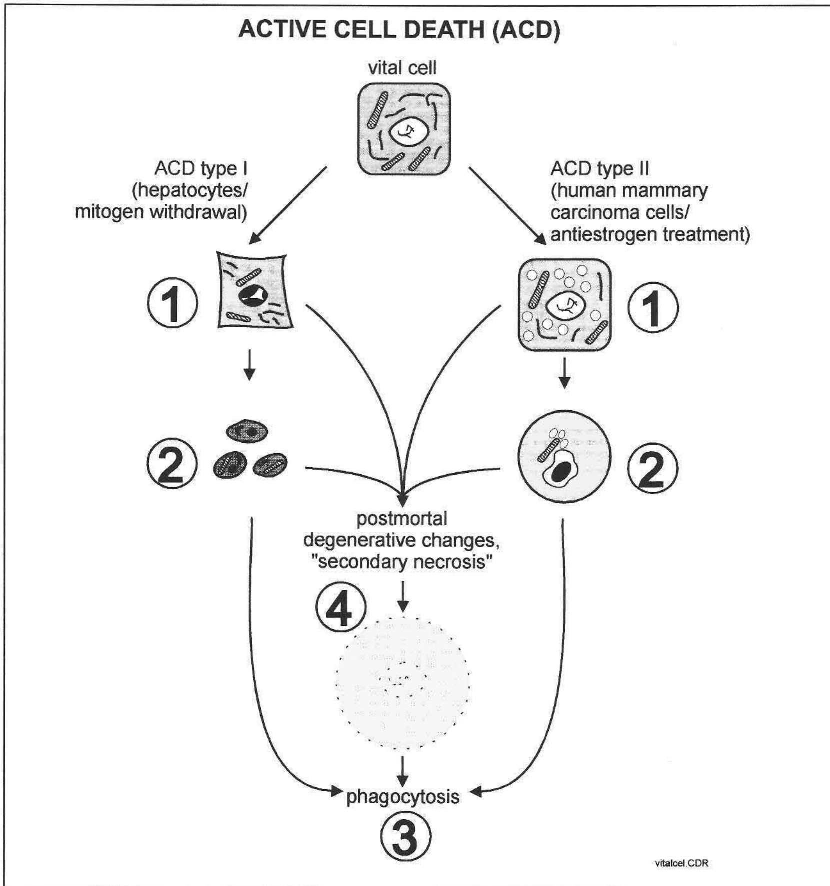

Fig. 1. Active cell death (ACD): Morphological sequence of type I (apoptosis) and type II (autophagic/lysosomal) ACD.

ACD Type I (apoptosis): 1. condensation of cytoplasm and of chromatin at the nuclear membrane to sharply delineated masses (often like crescents). 2. cell fragmentation into apoptotic bodies. 3. Phagocytosis (in vivo) and heterophagic degradation.
Note: autophagy/lysosomes do not play a distinct role early in apoptosis.

ACD Type II (autophagic/lysosomal cell death): 1. Autophagy: formation of autophagic vacuoles (AVs; open circles) and degradation of cytoplasmic constituents; 2. Pyknosis, single pyknotic mass in the center of the nucleus, nuclear envelope still intact, cytoplasm amorphous with few clusters of AVs and mitochondria. 3. Phagocytosis (in vivo) and final degradation.
4. Note: a cell may enter a given ACD pathway which, however, may not be completed and secondary necrosis ensues (see text for details).

doses of thioacetamide or dimethylnitrosamine first induced apoptosis and then necrosis in rat liver in vivo (Pritchard and Butler 1989, Ledda-Columbano et al. 1991). Secondly, *in vivo* massive physiological signals triggering ACD may overload the degradative machinery (autophagy, phagocytosis and heterophagic digestion of

cell fragments) which may result in lytic changes resembling necrosis (figure 1). Thus, in hepatocellular carcinoma produced in rats by longterm treatment with the non-genotoxic carcinogen nafenopin, a dramatic increase of cell death (initially mainly apoptosis) and tumor regression was triggered by withdrawal of nafenopin. With increasing time of withdrawal cancers developed large areas of hydropic and fatty degeneration and lytic cell death which was also reflected by a concomitant increase of glutamate dehydrogenase in serum. Such lesions were not found in tumors *during* nafenopin treatment indicating that the formation of lytic cell death was a secondary phenomenon, probably due to insufficiency of phagocytotic mechanisms in rapidly regressing tumors (Schulte-Hermann et al. 1995, Grasl-Kraupp et al. 1997). Likewise, in *cell cultures* dead cells — in particular upon detachment from the substrate — may undergo postmortal degenerative changes ('secondary necrosis' figure 1; Wyllie et al. 1980). Unfortunately, upon a given stimulus many cell types enter cell death asynchronously. Consequently, at a given time point all stages of active cell death as well as secondary necrosis may be present and render biochemical and molecular studies on *cell homogenates* very difficult or even impossible (Cejna et al. 1994).

Furthermore, in our experiments with MCF-7 cells 50 Kb DNA fragments as well as oligo-nucleosomes were also detected during non-active, *lytic* cell death. This may suggest that activation of the enzymes producing the regular pattern of DNA fragmentation is not restricted to ACD, although DNA degradation during lytic cell death is much more rapid than in the active one (24 hours vs. 3-5 days, resp.). Most probably membrane destruction results in an increase in cytoplasmic and nuclear Ca^{++} and Mg^{++} ions thereby activating DNAses (Walker at al. 1993, Cain et al. 1994). Likewise, necrosis of neuronal cells has been found to be associated with transient formation of oligonucleosomes (Collins et al. 1992). These observations emphasize current notions that certain patterns of DNA fragmentation (e.g. DNA ladders) should not be considered specific for the apoptotic mode of ACD.

Recently, techniques have been developed which allow detection of DNA fragments in situ on histological sections using DNA polymerase or terminal transferase to label DNA ends ('TUNEL' assay). Pyknotic nuclei of MCF-7 cells undergoing ACD exhibited DNA strand breaks as demonstrated histochemically in individual cells using the TUNEL technique (Sikorska, Walker, Bursch, unpublished observation). However, a positive result not necessarily is a reliable and specific indicator of active cell death or of apoptosis as exemplified by in vivo studies on rat liver. A positive response after applying the TUNEL technique was also obtained in nuclei of necrotic liver cells after intoxication with CCl_4 and even as a result of tissue autolysis (Grasl-Kraupp et al. 1995).

In conclusion, to date there is no single biochemical or molecular marker for detection of active cell death subtypes available. Therefore, morphological, biochemical and functional criteria applicable to the specific conditions under study (in vivo, in vitro, cell suspension or not, etc.) should be used in combination (table 1). Morphological methods are still required to identify cells undergoing active cell death, and electron-microscopy may be necessary for unequivocal identification. Detection of mRNAs or proteins if specific for active cell death in a given tissue, is an additional possibility but candidate genes or proteins generally applicable have not yet been found. As to the

Table 1. Morphological, Biochemical and Functional Criteria Helpful for Identification and Quantification of Active Cell Death:

Cytoplasmic and nuclear changes		
	Morphologically:	Light microscopy (H&E, Hoechst 33258 stain in vitro)
		Electron microscopy
	FACS:	Suspendable cells, isolated nuclei
DNA degradation		
	DNA ladders (not always positive, not specific)	
	TUNEL (not specific)	
Cytoplasmic degradation		
	Proteasome/ubiquitin (non-apoptotic types of ACD)	
	Autophagic/lysosomal activity (-//-)	
Functional		
	Induction by mitogen withdrawal	
	Cell rescue by survival factors	
	Receptor mediated	
Rates of cell death (in vivo)		
	Apoptotic index:	Determination requires information about duration of visible stages of ACD, circadiane rhythm, number of cell fragments ('apoptotic bodies') deriving from *one* cell upon its fragmentation.
		Technical requirements: standardized, equal thickness of sections, intensity of stain; control of histological counts for inter- and intra-individual deviations caused by investigator(s)

discrimination between ACD and necrosis, functional characteristics such as inhibition of ACD (i.e. rescue) by cell/tissue specific mitogens and survival factors may be helpful and have been widely used, both in vivo and in vitro (for review: Schulte-Hermann et al. 1995).

Quantification of Active Cell Death in vivo and Implications for Carcinogenesis

Quantitative determination of the incidence of cell death in vivo requires counting of histological tissue sections and good quality sections stained with HE are essential (Goldsworthy et al. 1996, Schulte-Hermann et al. 1995). However, the incidence of histological signs of cell death is not sufficient to assess the quantitative role of cell death in the kinetics of growth and involution of a given tissue. Rather, this approach requires determination of the frequency of cell death per unit time, i.e. death rate. Therefore we have estimated the duration of the histologically visible stages of apoptosis of individual hepatocytes in vivo (both in normal cells as in preneoplastic tissue) which was found to be about 3 hours (Bursch et al.1990). This short duration explains why apoptosis is relatively rarely observed in histological sections even in

states of considerable cell loss. Moreover, the incidence of apoptosis in the liver and probably in other tissue exhibits a circadiane rhythm. Thus, the feeding/fasting state of the animal profoundly modifies the incidence of apoptosis, yielding high rates at the end of the daily light (fasting) period, and low rates in the dark (feeding) phase (Schulte-Hermann et al. 1988, Grasl Kraupp et al. 1994,). Thus, if rodents are sacrificed in the morning according to laboratory routine, apoptotic rates in the liver may be minimal and any increase is due to a specific state or treatment of animals might be easily missed.

As to the implications of apoptosis for carcinogenesis, we have long been interested in the mechanisms of tumor promotion using rat liver as the major experimental model. In the liver, initiated cells can be detected as single cells or small foci of cells by immunocytochemical and histochemical means (Schulte-Hermann et al. 1990, 1995). In the early 80ies we discovered that these foci exhibit higher rates of cell proliferation than normal liver which, however, may be counterbalanced by apoptosis. These foci can be stimulated to grow by tumor promoters such as phenobarbital and many others (Schulte-Hermann et al. 1990, 1995). Closer analysis showed that tumor promoters have little effect on cell proliferation but their predominant effect is inhibition of apoptosis in the foci (i.e. survival factor activity), thereby allowing rapid accummulation of preneoplastic cells (Schulte-Hermann et al. 1982, 1990, Bursch et al. 1984). These findings showed that tumor promoters can act as survival factors for preneoplastic cells in vivo and provided a new approach to understand the mechanism of action of this class of risk factors. Even malignant cells in the liver were found to still depend on the presence of survival factors as exemplified by a dramatic increase in cell death and rapid regression of liver tumors upon withdrawal of nafenopin (see above). The occurrence of apoptosis in liver foci was subsequently confirmed and extended by other groups (Columbano et al. 1984; Garcea et al. 1989). In vitro studies by Kanter et al. (1984) showed that tumor promoters such as TPA (12-0-tetradecanoyl-13-0-phorbol acetate) and epidermal growth factor protect fibroblasts from cell death upon serum withdrawal.

More recently we have carefully determined rates of cell birth and death in various stages of cancer in the liver. It was found that both birth (α) and death (β) rates gradually increased from normal liver to putative preneoplastic foci to hepatocellular adenoma and to hepatocellular carcinoma, the net growth rate of tissue is determined by $\alpha - \beta$ (Grasl-Kraupp et al. 1997). (Pre)neoplastic cell populations (pn, tu) in general exhibit higher net growth rates than the tissue of origin (n*(ormal)*), namely $(\alpha_{pn,tu} - \beta_{pn,tu}) > (\alpha_n - \beta_n)$. As in rats, in human liver cancer birth and death rates were increased manyfold, indicating a species-independent phenomenon (Grasl-Kraupp et al. 1997). Likewise, in human pre-malignant and malignant colonic tumors rates of cell proliferation and active cell death were much higher than in normal tissue (although cell death rates in carcinoma were somewhat lower than in adenoma; Partik et al. 1997). The gradual increase of cell turnover during the sequential stages of carcinogenesis has important implications. Thus, the more cell turnover is accelerated, the greater should be the impact even of *small* imbalances between cell birth and death on cell number. Experimental evidence for this prediction has been provided: a 90 days period of food restriction results in an increase of apoptosis in preneoplastic liver foci

and in selective disappearance of the majority of these lesions (Grasl-Kraupp et al. 1994). Likewise, injection of TGF-ß1, one of the physiological death signals for normal hepatocytes, strongly enhanced apoptosis also in preneoplastic cells resulting in their selective elimination (Müllauer et al. 1996). A further important implication of increasing death rates has first been described by Moolgavkar and Luebeck on mathematical grounds, namely that cell clones may become extinct (Luebeck et al 1995). The probability of extinction of a clone is expressed by ß: α. Thus, if food restriction would cause an eradication of preneoplastic lesions (in other words: reduce the number of 'promotable' lesions), fewer tumors eventually should develop as compared to animals feed ad libitum during their life. We have experimentally provided prove for this prediction by showing that the food restriction indeed partially protects against carcinogenesis by subsequent treatment with the tumor promoter nafenopin (Grasl-Kraupp et al. 1994).

In conclusion, these findings clearly indicate that active cell death may well occur in malignant tumors. The current notion by some authors that cancers develop because of an inability of cells to undergo active cell death does not apply to the cancers studied here and probably not to others. Rather, it appears that preneoplastic and neoplastic cells are *more* susceptible to signals inducing proliferation or death than normal cells. Therefore, tumor promoters will induce selective (preferential) growth of (pre)neoplastic lesions. Conversely, anti-promotion (food restriction, TGF ß1, lack of survival factors due to promoter withdrawal) may cause selective regression by preferential increase of active cell death. These findings may be important for risk assessment of non-genotoxic carcinogens. Furthermore, more detailed knowledge of survival factors and death factors controlling birth and death rates of cells in tissue may provide new possibilities for selective induction of active cell death and thereby, for cancer prevention and therapy.

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