References

[1] Krammer, P.H. CD95(APO-1/Fas)-Mediated Apoptosis: Live And Let Die (ed. Frank J. Dixon). Advances in Immunology, 163-210, 1998.
[2] Peter, M.E. and Krammer, P.H. Mechanisms of CD95(APO-1/Fas)-mediated Apoptosis. Current Opinion in Immunology 10, 545-51, 1998.
[3] P.H. Krammer, Tumorimmunology Program, German Cancer Research Center, Heidelberg, Germany

19

Radiation response and apoptosis

M. Verheij. *Antoni van Leeuwenhoekhuis, Radiotherapy Department, Amsterdam, The Netherlands*

Apoptosis or programmed cell death is a distinct mode of cell destruction and represents a major regulatory mechanism for removing abundant and unwanted cells during embryonic development, growth, differentiation and normal cell turnover. Failure to eliminate cells that have been exposed to mutagenic agents has been associated with the development of cancer and resistance to anticancer therapy.

Ionizing radiation, like most chemotherapeutic agents, induces apoptosis in a wide variety of cell systems. The magnitude of this response, however, depends to a large extent on the cell type and the dynamic balance between survival and apoptosis-promoting signals. An essential step in the execution phase of the apoptotic death program involves the sequential cleavage and activation of a group of cysteine proteases (caspases). Radiation may activate this caspase cascade in different subcellular compartments and by different mechanisms. Upon its activation by DNA-damaging agents, the tumor suppressor p53 can act as a direct transcriptional regulator of bax, a pro-apoptotic member of the Bcl-2 family. P53-dependent upregulation of death receptor/ligand systems provides another link between radiation-induced DNA damage and the apoptotic machinery. At the mitochondrial level radiation-induced reactive oxygen intermediates have been shown to mediate the release of cytochrome c and subsequent caspase activation. Finally, plasma membrane-derived lipid second messengers, activating stress-induced signal transduction pathways may also target the caspase cascade.

The potential radiobiological and clinical relevance of radiation-induced apoptosis is illustrated by several lines of experimental evidence: (1) overexpression of apoptosis-promoting or -suppressing genes modifies radiation-induced clonogenic cell survival and radiosensitivity, (2) the cellular propensity to undergo apoptosis is maintained throughout fractionated radiation schedules, (3) in certain tumors pretreatment levels of apoptosis have been shown to predict for clinical outcome after radiotherapy.

In summary, radiation-induced apoptosis has been a focus of intense research during the last decade. Its recognition as a significant component of radiation-induced cell death and potential co-determinant of radiosensitivity has initiated several lines of research aimed at modulating the apoptotic response in both tumor and normal cells in order to increase the radiotherapeutic ratio.

20

More than one way to die: apoptosis and necrosis induced by death domain receptors

P. Vandenabeele. *University of Gent, Flanders Interuniversity Institute for Biotechnology, Department of Molecular Biology, Gent, Belgium*

Apoptosis and necrosis are two distinct forms of cell death. In an in vitro cell culture model system of death domain receptor induced cell death, we have compared morphological and signal transduction events occurring during Fas-ligand induced apoptosis and TNF-induced necrosis in the same cellular context of the L929sA fibrosarcoma cell line. Caspases are indispensable as initiators and effectors of apoptotic cell death and are involved in many of the morphological and biochemical features of apoptosis. Major changes in mitochondrial membrane integrity and release of proapoptotic factors, such as cytochrome c from the mitochondrial intermembrane space, play an important sensor and amplifying role during apoptotic cell death. Necrosis is not correlated with active caspases, cytochrome c release or internucleosomal DNA fragmentation. Principal elements of necrosis include mitochondrial oxidative phosphorylation, reactive oxygen production, and non-caspase proteolytic cascades depending on serine proteases, calpains, or cathepsins. Inhibition of the classical caspase-dependent apoptotic in several cell lines pathway leads to necrotic cell death. Thus, the same cell death stimulus can result either in apoptotic or necrotic cell death, depending on the availability of activated caspase. Therefore, death domain receptors may initiate an active caspase-independent necrotic signaling pathway. Also the differential interrelation between apoptotic and necrotic cells and macrophages will be discussed.

21

Apoptosis in the normal and malignant colon

J.A. Hickman[1], C.S. Potten[2], M. Pritchard[3], A. Jackman[4], F. Meyer-Losic[1]. *[1] Centre de Recherches de Suresnes, Institut de Recherches Servier, Suresnes, France; [2] Christie Hospital, Cell Biology, Manchester, England; [3] Liverppool University, Medicine, Liverpool, England; [4] Institute of Cancer Research, Centre for Drug Discovery, Sutton, England*

The question is intruiging as to why the normal epithelia of the gut are very sensitive to chemotherapeutic drugs, whilst carcinomas of the colon are resolutely resistant to drugs like 5-fluorouracil (5-FU) or Tomudex. Our experiments suggest that the "survival threshold" of normal epithelial cells is set by a number of key genes which control apoptosis and progress through the cell cycle. This threshold determines how easy or difficult it is for a cell to die after drug-induced damage. For example, 5-FU toxicity to mouse small intestinal epithelia is lost in an animal in which the tumour suppressor gene p53 has been deleted. Both functions of p53, as a pro-apoptotic protein and as an initiator of cell cycle delay, via p53-stimulated expression of the cyclin-dependent kinase inhibitor p21,are required for the expression of toxicity. Loss of p53 in colon tumors indicates a poor prognosis. Mice in which p21 has been deleted show an attenuated response to 5-FU.

Interestingly, the pure thymidylate synthase inhibitor Tomudex, considered to have mechanistic similarities to 5-FU, and which is used to treat colon carcinoma, has a p53-independent mode of action. Gut epithelia from mice of different strains differed profoundly in their response to Tomudex, suggesting that it is possible to use genetic methods to estimate the numbers of genes which set the "survival threshold" and to isolate those responsible for p53-independent cells death. These genes are likely to be altered in late stage p53 mutated/deleted tumours and to provide the gateway to effective therapies of an otherwise inherently chemo-resistant tumour.

References

[1] Pritchard, D.M., Bower, L., Potten, C. S. Jackman, A. L. and Hickman, J.A. (2000) The Importance of Apoptosis and p53 Expression for the Strain-Dependent Intestinal Toxicities Induced by Raltitrexed (ZD1694, Tomudextm) In Balb/C And DBA/2 Mice. Clin Cancer Res, 6: 4389-95.

22

Regulation of p73 in cell death

V. De Laurenzi, D. Barcaroli, M. Ranalli, G. Melino. *IDI-IRCCS, Biochemistry Lab., c/o Dept. Experim. Medicine, University of Rome Tor Vergata; 00133 Rome, Italy*

The p53-homologue p73, has been mapped to a region (1p36.33) which is frequently deleted in neuroblastomas, suggesting that its alterations may play a role in the development of tumours of the nervous system. However, unlike p53, mutant p73 has rarely been found in human tumours. Few studies have directly investigated functions of p73, and their activities have been largely assumed based on their structural similarities with the p53 homologue. p73 has indeed been shown to induce apoptosis when transfected into cells. Here we show that:

(1) p73 is expressed as distinct forms at the C-terminus (spliced forms, α, β, γ, δ, ε and ζ) and N-terminus (two distinct promoters for TA-p73 and Delta-N-p73), which show different abilities to homo/hetero-dimerize and transactivate target promoters.
(2) DNA damaging agents, through MLH1 and c-Abl, increase the half-life of p73. This pathway is independent and parallel to p53, being relevant for cancer development, progression, and therapy.
(3) p53 regulates transcriptionally Delta-N-p73, which in turn functionally inactivates p53 (loop).
(4) p73δ is induced during keratinocyte differentiation, while p63/ΔNp63 are repressed. Both p73δ/p63 transactivate differentiation specific promoters.
(5) p73 trigger neuronal differentiation: p73 expression is upregulated during neuroblastoma differentiation. Overexpression of p73 (not A156V mutants or p73Δ84) trigger neuronal differentiation (regulating MYCN, NCAM, pRB). Dominat negative 73Δ84 blocks NCAM transactivation induced by retinoids.

In conclusion, p73 seems involved both in tumour suppression and development.