



Editorial

Back to the future: The role of cytochrome *c* in cell death

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Recent developments have placed the mitochondria in the focus of apoptosis research (Orrenius *et al.*, 1997 and references therein). Increasing evidence supports the hypothesis that factors released from these organelles, notably cytochrome *c*, can serve to activate cytoplasmic caspases and thereby trigger the execution phase of apoptosis (Liu *et al.*, 1996). Similarly, microinjection of cytochrome *c* into various cell types has been found to cause cell death by apoptosis (Li *et al.*, 1997a; Zhivotovsky *et al.*, 1998). Whether cytochrome *c* release from mitochondria is preceded by mitochondrial permeability transition and loss of the transmembrane potential is still a controversial issue (Kroemer, 1997; Yang *et al.*, 1997; Kluck *et al.*, 1997). It is also unknown if all caspases are equally sensitive to cytochrome *c*-mediated activation.

However, the observations that alterations in mitochondrial function and release of cytochrome *c* may be of importance for cell death are not novel. Before the epoch of apoptosis, cell death was studied quite intensively in radiation biology, because it is the ultimate effect of ionizing radiation. Studies of radiation-induced alterations in the cellular bioenergetic machinery was one of the most actively developing areas in radiobiology in the 1950s and 1960s (Ashwell and Hickman, 1952; vanBekkum, 1957; Scaife, 1964; Manoilo, 1968). Cell death was divided into reproductive cell death (dependent on the mitotic cycle) and interphase cell death (Okada, 1970). The latter was early regarded as a classic example of apoptosis (Hanson, 1979; Yamada and Ohyama, 1988). More recently, it has become clear that radiation-induced reproductive cell death often also occurs by apoptosis. Furthermore, tissues were divided into radiosensitive (thymus, spleen, small intestine, etc.) and radioresistant (liver, heart, brain, etc.) according to the early appearance of cell death after irradiation (Bacq and Alexander, 1961).

During the search for mechanisms of early massive cell death in radiosensitive tissues in the 1950s, suppression of oxidative phosphorylation in mitochondria from thymus and spleen was described (Ashwell and Hickman, 1952). This phenomenon was not seen in the mitochondria from radioresistant tissues (Hanson and Mytareva, 1967). The suppression of oxidative phosphorylation was observed 30–60 min after whole-body X-irradiation of rats with

relatively low doses of radiation (50–100 cGy) (vanBekkum, 1957). In radiosensitive tissues, the suppression of oxidative phosphorylation was associated with the formation of pyknotic nuclei (vanBekkum *et al.*, 1964). Using selective dyes, it was found that there was a retardation of electron transport between cytochromes *b* and *c* in thymus mitochondria which exhibited lower levels of cytochrome *c* after irradiation (Scaife, 1964). Thus, the radiation lesion was believed to be due to the looser binding of cytochrome *c* to the mitochondrial inner membrane after X-irradiation.

This observation was in accordance with the previous finding that addition of exogenous cytochrome *c* could stimulate oxidative phosphorylation in mitochondria isolated from radiosensitive, but not from radioresistant, tissues of irradiated rats (Manoilo and Hanson, 1964; Hanson and Mytareva, 1967). Loss of cytochrome *c* was not a result of its simple escape from the mitochondria, since additional washing of the mitochondrial fraction with isotonic buffer did not increase the 'cytochrome *c*' effect (van Bekkum, 1957). Neither did *in vitro* irradiation of isolated mitochondria cause increased enzyme release (Scaife and Hill, 1963). Thus, it was suggested that the perturbation of mitochondrial electron transfer in radiosensitive tissues was based on a controlled release of cytochrome *c* from the mitochondria and the appearance of the hemoprotein in the cytosol (Scaife, 1964, 1966). It is interesting to note that irradiation damage to cytochrome *c* *in vitro* was appreciably less when it was in the fully reduced state rather than partially oxidized or undergoing alternative oxidation-reduction changes. In strong support of these earlier observations are recent reports on cytochrome *c* release from mitochondria in cells undergoing radiation-induced cell death (Kharbanda *et al.*, 1997; Chauhan *et al.*, 1998) and on the importance of the redox state of cytochrome *c* during caspase activation in cytosolic extracts (Hampton *et al.*, 1998).

It is also of interest that only a modest decrease in ATP levels was observed in thymus, spleen and ascites tumor cells following irradiation (Scaife, 1966). This observation suggested that the loss of functional cytochrome *c* from the mitochondria was not large enough to markedly depress ATP production by the mitochondria or that this loss could be compensated for by glycolytic phosphorylation systems. In fact, several recent observations support the idea that a combination of cytochrome *c* release and maintenance of a sizable intracellular ATP pool is required for the execution of the apoptotic suicide program (Tsujiyama, 1997; Nicotera and Leist, 1997). In recent reviews, Reed (1997) and Schendel (1998) discuss two pathways of cell killing by cytochrome *c* released from the mitochondria. One of them involves activation of the caspase cascade by the

interaction of the released hemoprotein with Apaf-1 and pro-caspase-9 in the cytosol, while the other one is related to the retardation of mitochondrial electron transport and subsequent impairment of ATP production and promotion of the generation of reactive oxygen species. This second pathway is very similar to the one described by Scaife (1966) many years ago.

Thus, the mitochondria and their release of cytochrome *c* was in the focus of cell death research more than thirty years ago. The mechanism then was believed to be restricted to impairment of ATP production and enhanced generation of reactive oxygen species. The recent developments have added another mechanism, i.e. caspase activation by the released cytochrome *c*, which is now believed to be critical for the apoptotic process (Zou *et al*, 1997; Li *et al*, 1997). Future research will reveal whether there are additional mechanisms by which the mitochondria can kill their host cells.

References

- Ashwell G and Hickman J (1952) Effects of X-irradiation upon the enzyme systems of the mouse spleen. *Proc. Soc. Exptl. Biol. Med.* 80: 407–413
- Bacq ZM and Alexander P (1961) *Fundamental radiobiology*. Acad. Press, NY - L
- Chauhan D, Pandey P, Agata A, Teoh G, Krett N, Halgren R, Rosen S, Kufe D, Kharbanda S and Anderson K (1998) Cytochrome *c*-dependent and -independent induction of apoptosis in multiple myeloma cells. *J. Biol. Chem.* 272: 29995–29997
- Hampton MB, Zhivotovsky BD, Slater AFG, Burgess DH and Orrenius S (1998) Importance of the redox state of cytochrome *c* during caspase activation in cytosolic extracts. *Biochem. J.* 329: 95–99
- Hanson KP (1979) Molecular mechanisms of interphase cell death. *Radiobiology* 19: 814–820 (In Russian)
- Hanson KP and Mytareva LV (1967) Mechanisms of effects of ionizing radiation on oxidative phosphorylation in animals. *Proc. Acad. Sci. (Estonia)* 16: 80–87
- Kharbanda S, Pandey P, Schofield L, Israels S, Roncinske R, Yoshida K, Bharti A, Yuan ZM, Saxena S, Weichselbaum R, Nalin C and Kufe D (1997) Role for Bcl-xL as an inhibitor of cytosolic cytochrome *c* accumulation in DNA damage-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 94: 6939–6942
- Kluck RM, Bossy-Wetzel E, Green DR and Newmeyer DD (1997) The release of cytochrome *c* from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science* 275: 1132–1136
- Kroemer G (1997) Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution. *Cell Death Differ.* 4: 443–456
- Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X (1997) Cytochrome *c* and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479–489
- Li F, Srinivasan A, Wang Y, Armstrong RC, Tomaselli KJ and Fritz LC (1997a) Cell-specific induction of apoptosis by microinjection of cytochrome *c*. Bcl-x_L has activity independent of cytochrome *c* release. *J. Biol. Chem.* 272: 30299–30305
- Liu X, Kim NC, Yang J, Jemmerson R and Wang X (1996) Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome *c*. *Cell* 86: 147–157
- Manoilov SE (1968) Primary mechanisms of biological effects of ionizing radiation. *Meditsina, Leningrad*
- Manoilov SE and Hanson KP (1964) Effect of exogenous cytochrome *c* on oxidative phosphorylation in mitochondria from tissues isolated from irradiated animals. *Vopr. Medic. Chem.* 10: 410–416 (In Russian)
- Nicotera P and Leist M (1997) Intracellular ATP, mitochondrial function and the decision between apoptosis and necrosis. *Cell Death Differ.* 4: 435–442
- Okada S (1970) In: *Radiation Biochemistry* (Altman KI, Gerber GB and Okada S, eds.) Vol. 1, Academic Press, New York.
- Orrenius S, Burgess DH, Hampton MB and Zhivotovsky B (1997) Mitochondria as the focus of apoptosis research. *Cell Death Differ.* 4: 427–428
- Reed J (1997) Cytochrome *c*: Can't live with it - Can't live without it. *Cell* 91: 559–562
- Scaife JF (1964) The nature of the radiation-induced lesion of the electron transport chain of thymus mitochondria. *Canad. J. Biochem.* 42: 431–434
- Scaife JF (1966) The effect of lethal doses of X-irradiation on the enzymatic activity of mitochondrial cytochrome *c*. *Canad. J. Biochem.* 44: 433–439
- Scaife JF and Hill B (1963) Uncoupling of oxidative phosphorylation by ionizing radiation. II The stability of mitochondrial lipids and cytochrome *c*. *Canad. J. Biochem.* 41: 1223–1227
- Schendel S, Montal M and Reed JC (1998) Bcl-2 family proteins as ion-channels. *Cell Death Differ.* 5: (in press)
- Tsujimoto Y (1997) Apoptosis and necrosis: Intracellular ATP as a determinant for cell death modes. *Cell Death Differ.* 4: 429–434
- vanBekum DW (1957) The effect of X-rays on phosphorylation *in vivo*. *Biochim. Biophys. Acta* 25: 487–493
- vanBekum DW, deVries MJ and Klown HM (1964) Biochemical and morphological changes in lymphatic tissues following partial-body irradiation. *Internat. J. Radiat. Biol.* 8: 395–401
- Yamada T and Ohyama H (1988) Radiation-induced interphase death of rat thymocytes is internally programmed (apoptosis) *Int. J. Radiat. Biol.* 53: 65–75
- Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng T-I, Jones DP and Wang X (1997) Prevention of apoptosis by Bcl-2: Release of cytochrome *c* from mitochondria blocked. *Science* 275: 1129–1131
- Zhivotovsky B, Orrenius S, Brustugun OT and Doskeland SO (1998) Injected cytochrome *c* induces apoptosis. *Nature* 391: 449–450
- Zou H, Henzel WJ, Liu X, Lutschg A and Wang X (1997) Apaf-1, a human protein homologous to *C. Elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. *Cell* 90: 405–413