

Ceramide in malignant tumors

Review Article

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Summary

The lipid ceramide is widely recognized as being central for the mediation of the cellular stress response and the regulation of apoptosis in many cells. Ceramide has been demonstrated to be required for the cellular response to stress stimuli such as ionizing radiation, chemotherapy, UVA-light, heat, CD95 and TNF receptor ligation, reperfusion injury, infection with some pathogenic bacteria and viruses and developmental programmed cell death of oocytes. We recently proposed a comprehensive model for the molecular function of ceramide. This model suggests that ceramide self-associates to ceramide-enriched membrane microdomains that subsequently fuse to larger macrodomains and platforms. These ceramide-enriched platforms serve to transmit signals via receptors into the cell, e.g. by reorganizing and concentrating receptors and signaling molecules within a defined area of the cell membrane. Ceramide-enriched membrane platforms might also mediate the cellular effects of ionizing radiation, heat or cytostatic drugs, providing a rationale for the very high radio-resistance of cells lacking the acid sphingomyelinase, which endogenously generates ceramide from sphingomyelin. Translation of these concepts into tumor biology suggests that an inhibition of acid sphingomyelinase expression or function, confers resistance of the tumor against radiation and/or chemotherapy, while an increase of acid sphingomyelinase activity might open an avenue to novel therapy concepts.

I. Introduction

The generation of ceramide by rapid sphingomyelinase-mediated hydrolysis of plasma membrane sphingomyelin was first shown by Kolesnick and Paley (1987) to play a role in cellular signaling. Studies of the last years identified a comprehensive mechanism for the cellular functions of ceramide (Grassmé et al, 1997, 2001a, b, 2002, 2003a, b, Cremesti et al, 2003). Membranes of mammalian cells are mainly composed of glycosphospholipids, (glyco-) sphingolipids, and cholesterol. Glycosphingolipids tightly associate with each other by hydrophilic interactions between their head groups resulting in a lateral organization of these lipids (for reviews see Simons and Ikonen, 1997; Brown and London, 1998). However, in order to separate from other phospholipids in the cell membrane and to form distinct domains, void spaces between the large and bulky sphingolipid molecules must be filled. This function is primarily performed by cholesterol (Simons and Ikonen, 1997). Cholesterol interacts with sphingolipids via hydrophilic interactions between its hydroxy-group and the headgroups of the sphingolipids, and via hydrophobic interactions between the cholesterol ring system and the

sphingosine-moiety of sphingolipids. The tight homophilic interaction of sphingolipids and the association with cholesterol results in a firm lateral organization of these lipids, leading to spontaneous segregation from other membrane lipids and the formation of discrete membrane domains. These domains are characterized by a liquid-ordered or even gel-like phase (Simons and Ikonen, 1997; Brown and London, 1998). The tight packing of lipids in these membrane domains renders them relatively resistant to detergents and thus, they were termed detergent insensitive glycosphingo-lipid-enriched membrane domains. Moreover, since one model suggested that these structures float in the ocean of other membrane lipids, they were shortly referred to as rafts (Simons and Ikonen, 1997). The term raft will be used in the current overview to describe small, distinct glycosphingolipid- and cholesterol-enriched membrane domains that are constitutively present in the cell membrane. Here, we provide a mechanistic model of how small rafts are transformed into large signaling units in the cell membrane that serve to transmit stress signals into the cell. Furthermore, we discuss the function of distinct membrane domains in response to cellular stress, the induction of

apoptosis, and the development and treatment of malignant tumors.

II. Ceramide-enriched membrane platforms

We have shown in the recent years that the generation of ceramide in the cell membrane is capable to transform small rafts into large membrane platforms that facilitate the transmission of signals into the cell (**Figure 1**) (Grassmé et al, 2001a, b, 2002, 2003a, b; Cremesti et al, 2001). Mammalian cells utilize three distinct types of sphingomyelinases to generate ceramide through hydrolysis of sphingomyelin. Sphingomyelinases are characterized by their pH optimum and thus, were termed acid, neutral and alkaline sphingomyelinases. Numerous studies revealed a signaling function of the acid and neutral sphingomyelinase (for review see Goni and Alonso, 2002), while a similar role of the alkaline sphingomyelinase remains to be defined.

In addition to ceramide generation by hydrolysis of sphingomyelin, ceramide can be also synthesized *de novo* via a pathway that is regulated by the enzymes serine-palmitoyl-transferase and ceramide synthase (for review see Goni and Alonso, 2002).

We have recently suggested a novel mechanism of how ceramide functions in cellular signal transduction.

Studies employing the CD95 receptor and the CD40 receptor indicated that stimulation of these receptors through physiological ligands or stimulatory antibodies results in an activation of the acid sphingomyelinase within seconds (**Figure 1**) (Kirschnek et al 2000, Paris et al 2000; Grassmé et al, 2001, 2002; Cremesti et al, 2001). Activation of the acid sphingomyelinase is accompanied with translocation of the acid sphingomyelinase from an intracellular compartment onto the cell surface (**Figures 2 and 3**) (Grassmé et al, 2001a, b 2001). Although not proven at present, we assume that the acid sphingomyelinase is stored within small, intracellular vesicles that are mobilized and fuse with the cell membrane upon cellular stimulation. The fusion of these vesicles results in exposition of the acid sphingomyelinase on the outer leaflet of the cell membrane. Once on the surface acid sphingomyelinase seems to preferentially localize within rafts (**Figure 3**). There, it consumes sphingomyelin and generates ceramide in the outer leaflet of the cell membrane. The generation of ceramide is the critical event required to transform small rafts of resting cells into a large signaling unit. The driving force of this transformation is the endogenous tendency of ceramide to aggregate and to spontaneously fuse small rafts into large ceramide-enriched membrane platforms (for review see Kolesnick et al, 2000).

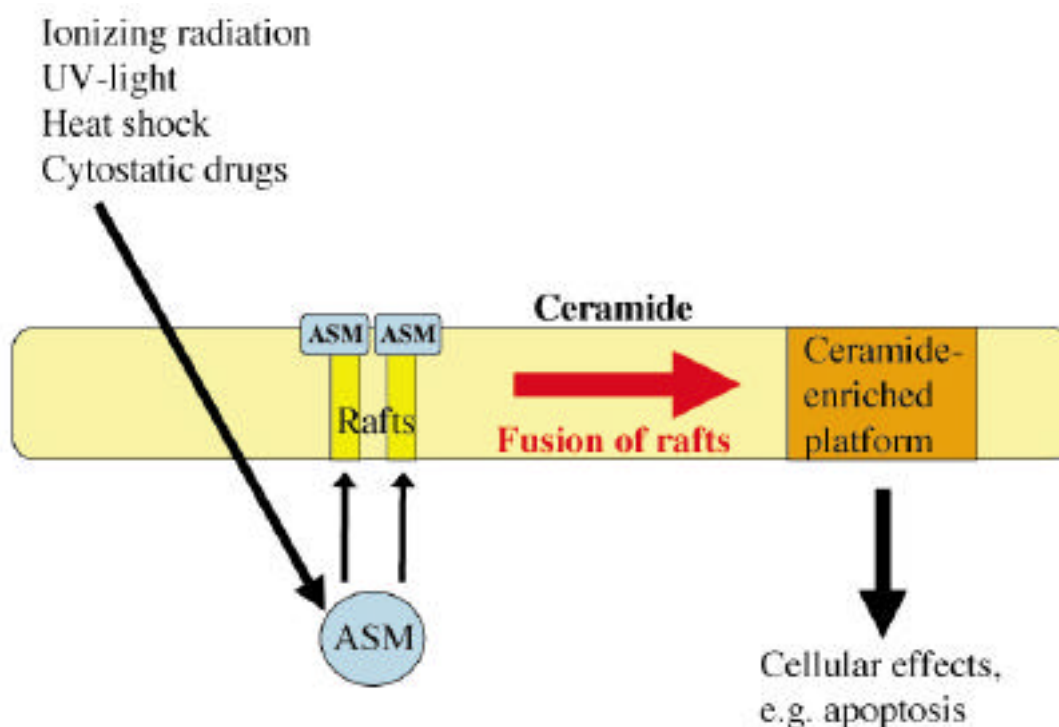


Figure 1: Model of raft formation and function

The model suggests that different stimuli including ionizing radiation, heat or chemotherapeutic drugs activate the acid sphingomyelinase and induce a translocation of the acid sphingomyelinase onto the extracellular leaflet of the cell membrane. The release of ceramide from sphingomyelin in the cell membrane results in the formation of small ceramide-enriched membrane microdomains that fuse to large, ceramide-enriched macrodomains. These platforms serve the transmission of the stress signal into the cell.

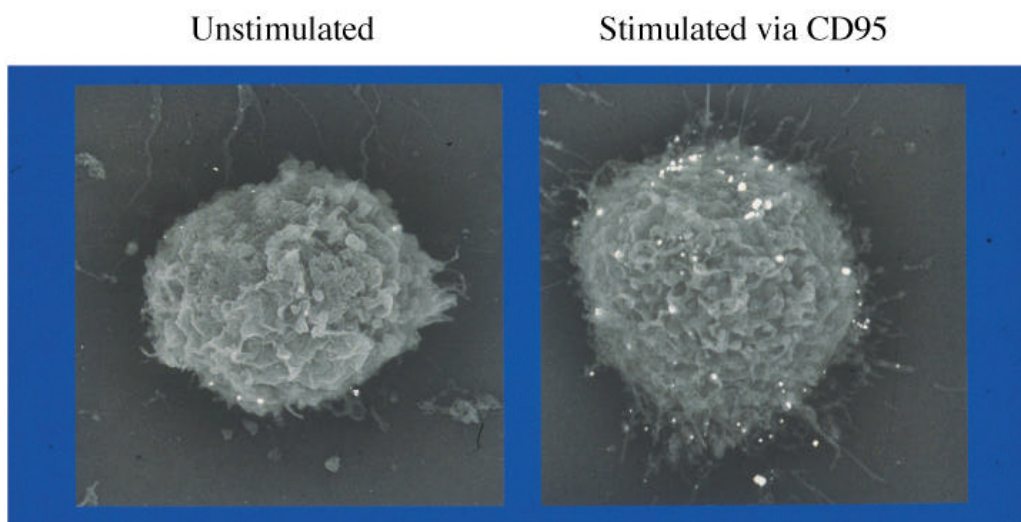


Figure 2: Acid sphingomyelinase translocates onto the surface of activated cells

Stimulation of JY B cells via CD95 triggers a translocation of the acid sphingomyelinase onto the extracellular leaflet of the cell membrane. Acid sphingomyelinase was visualized with a gold-coupled antibody that appears in the scanning electron microscopy analysis as white dots. The data indicate a distinct distribution pattern of the acid sphingomyelinase on the cell surface upon stimulation. Printed with permission of the J.B.C.

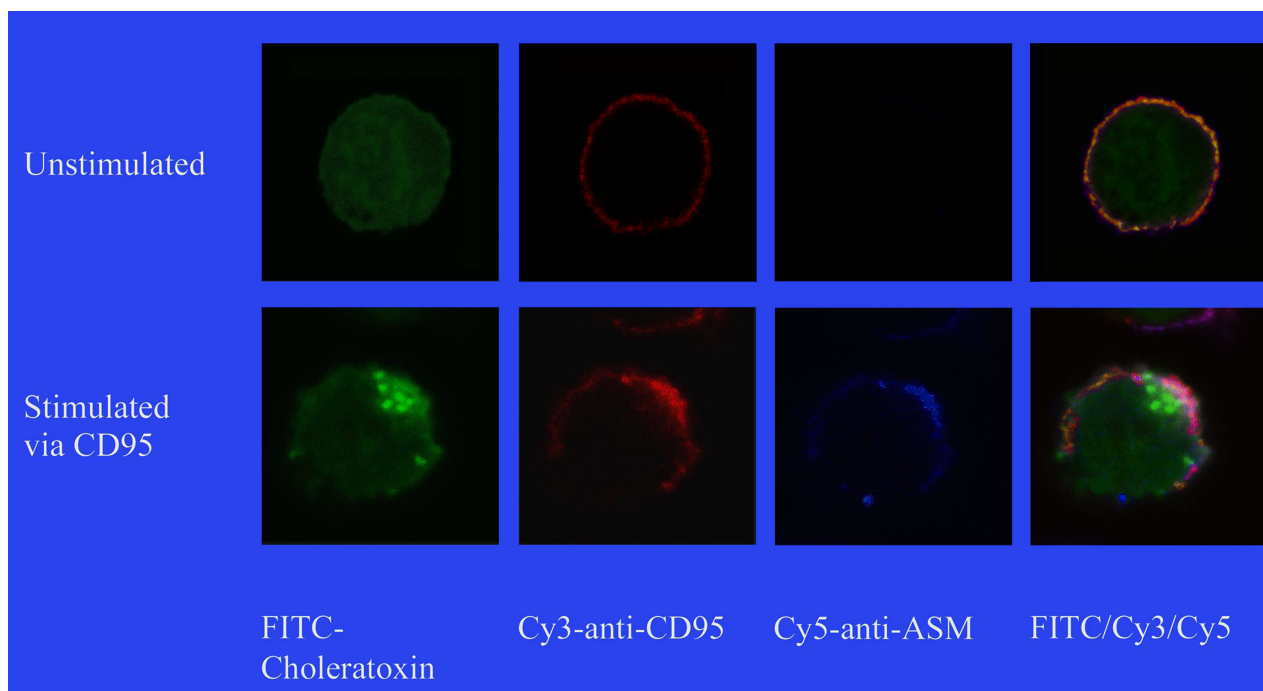


Figure 3: Acid sphingomyelinase mediates clustering of CD95

Lymphocytes were stimulated for 2 minutes via CD95, fixed and stained with FITC-coupled cholera toxin, that binds to the raft marker ganglioside GM1, Cy3-labelled CD95 and Cy5-coupled anti-acid sphingomyelinase antibodies. The results demonstrate clustering of CD95 and a co-localization of the clustered receptor with acid sphingomyelinase and cholera toxin. The latter suggests a clustering of CD95 and the acid sphingomyelinase in membrane rafts. Printed with permission of the J.B.C.

The formation of these large ceramide-enriched membrane platforms was shown *in vivo* for lymphocytes, fibroblasts, hepatocytes and epithelial cells (Kirschnek et al, 2000, Paris et al, 2000; Grassmé et al, 2001a, b, 2003). These findings were also confirmed on artificial, phosphatidylcholine /sphingomyelin-composed unilamellar membranes that were locally exposed to immobilized sphingo-

myelinase (Nurminen et al, 2002). The latter studies indicated that the generation of ceramide even in artificial membranes is sufficient to form large membrane platforms. In addition to triggering the fusion of rafts into large membrane platforms, ceramide also alters the composition of these membrane domains since the accumulation of ceramide results in an exclusion of

cholesterol from ceramide-enriched membrane platforms (Megha and London, 2003).

III. Ceramide-enriched membrane platforms and apoptosis

Ceramide-enriched membrane platforms promote the aggregation/clustering of receptor molecules, a phenomenon that has been best studied for CD95 and CD40 (Grassmé et al, 2001a, b, 2002; Cremesti et al, 2001). Studies on CD95 indicated that clustering occurs in many different cell types including lymphocytes, phagocytic cells, granulosa cells of the ovary, epithelial cells, fibroblasts, hepatocytes, and thymocytes (Fanzo et al, 2003). Clustering of the receptor in ceramide-enriched membrane platforms was shown to function as a mechanism to amplify signaling of this receptor approximately 100-fold. These studies further indicated that stimulation of CD95 in cells lacking the acid sphingomyelinase, which is essentially required to form ceramide-enriched membrane platforms upon receptor stimulation, results in only a very weak recruitment of FADD to CD95 and in a very limited activation of caspase 8 to an extent of less than 1% compared with complete activation of caspase 8 (Grassmé et al, 2003b). Acid sphingomyelinase-deficient cells also failed to activate caspase 3 and to undergo apoptosis. Transfection of these cells with acid sphingomyelinase or supplementation with natural C₁₆-ceramide was sufficient to restore clustering of CD95 after activation. Consequently, significant recruitment of FADD to CD95 and complete activation of caspase 8 was recovered and permitted sufficient activation of caspase 3 and the induction of apoptosis (Grassmé et al, 2003b). Therefore, we suggest that CD95 engages the acid sphingomyelinase pathway through a primary very weak and transient activation of caspases that is sufficient to induce surface translocation and activation of the acid sphingomyelinase. Acid sphingomyelinase finally mediates the formation of ceramide-enriched membrane platforms. At present it is unknown how other receptors, e.g. CD40, which are not coupled to caspases, are linked to the acid sphingomyelinase pathway.

These data indicate that clustering of CD95 in ceramide-enriched membrane platforms functions as an amplification mechanism that is most likely based on a high local density of receptor molecules in a small area of the cell membrane, permitting oligomerization of the receptor molecules. In addition, ceramide-enriched membrane platforms might serve to actively recruit signaling molecules and to bring these molecules in close contact to the activated receptor. This assumption is consistent with the recent findings that FADD and caspase 8 translocate into the detergent-insensitive membrane fraction upon cellular stimulation via CD95 (Scheel-Toellner et al, 2002). Moreover, the accumulation of ceramide might facilitate the exclusion of molecules from those platforms that may negatively interfere or even inhibit signaling via CD95.

The notion that the acid sphingomyelinase is central for the induction of apoptosis via CD95 is consistent with

previous *in vitro* and *in vivo* findings. It was demonstrated that *ex vivo* splenocytes or hepatocytes from acid sphingomyelinase knock-out mice were resistant to the induction of apoptosis by CD95 or TNF-receptor stimulation (Kirschnek et al, 2000; Paris et al, 2000; Garcia-Ruiz et al, 2003). Stimulation via the TNF-receptor has been previously shown to activate the acid sphingomyelinase and to release ceramide (Schütze et al, 1992). More important, *in vivo* data demonstrated that acid sphingomyelinase-deficient mice tolerated intravenous injection of agonistic anti-CD95 antibodies or TNF that usually induce acute hepatic failure (Garcia-Ruiz et al, 2003). These studies emphasize the *in vivo* significance of the acid sphingomyelinase for CD95- and TNF-receptor-mediated apoptosis. Further, human B-lymphocytes or fibroblasts from Niemann-Pick disease type A patients that suffer from an inborn deficiency of ASM failed to undergo apoptosis upon ligation of the CD95 receptor (Gulbins et al, 1995; DeMaria et al, 1998, Grassmé et al, 2001a). The susceptibility of lymphocytes and hepatocytes to CD95-triggered apoptosis was restored by reexpression of the acid sphingomyelinase or addition of natural C₁₆-ceramide to acid sphingomyelinase-deficient cells.

In summary, ceramide-controlled platform formation might function as a sorting device for certain receptor molecules that finally mediates amplification of signaling.

However, we would like to point out that these data do not exclude an intracellular function of the acid sphingomyelinase and ceramide, e.g. by reorganization of intracellular membranes or direct binding to and stoichiometric regulation of proteins.

A. Ceramide and ionizing radiation

Most data on the function of the acid sphingomyelinase in tumor biology have been published for the cellular effects of ionizing radiation (Haimovitz-Friedman et al, 1994; Santana et al, 1996; Pena et al, 2000; Paris et al, 2001; Garcia-Barros et al, 2003). Ionizing radiation activates the ASM within seconds to minutes in the plasma membrane of irradiated cells, resulting in a rapid release of ceramide (Haimovitz-Friedman et al, 1994). Preliminary data from our laboratory on glioma cells indicate that ceramide generated upon radiation forms large membrane platforms (**Figure 4**), very similar to those observed upon stimulation via CD95. Activation of ASM, release of ceramide, and the formation of ceramide-enriched membrane platforms are central for the induction of apoptosis by radiation as evidenced by the following data: Mature B cells, endothelial and mesothelial cells, or embryonic fibroblasts of acid sphingomyelinase-deficient mice were resistant to the induction of apoptosis by ionizing radiation, whereas cells expressing the acid sphingomyelinase rapidly died (Santana et al, 1996; Pena et al, 2000; Paris et al, 2001; Garcia-Barros et al, 2003). Recent experiments on the effects of ionizing radiation to the central nervous system confirmed the resistance of acid sphingomyelinase-deficient endothelial cells *in vivo* (Pena et al, 2000; Li et al, 2003). These studies reported the remarkable finding that endothelial cells lacking acid

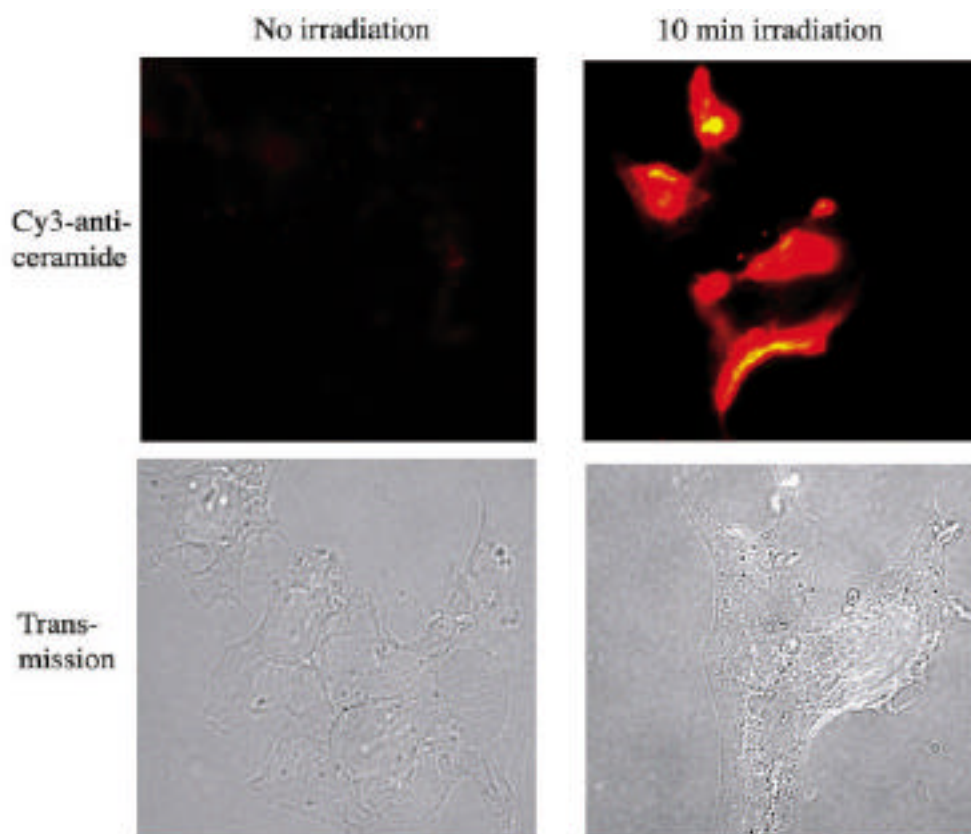


Figure 4: Radiation of glioma cells results in the formation of ceramide-enriched membrane platforms

LN229 glioma cells were radiated with 12 Gy and fixed 10 min after radiation. Ceramide on the cell surface was visualized by staining the cells with a Cy3-coupled anti-ceramide-antibody and analysed by fluorescence microscopy.

sphingomyelinase resisted radiation doses up to 40 Gy, while endothelial cells in normal C57Bl/6 or C3H/HN mice responded with apoptosis within the first 12 hours after radiation. Further studies on cells derived from Niemann-Pick Disease Type A patients proved the function of the acid sphingomyelinase for ionizing radiation-induced cell death. Retransfection of the acid sphingomyelinase into these cells or supplementation of natural C₁₆-ceramide restored radiation-induced apoptosis demonstrating the central role of the acid sphingomyelinase and, even more important, the role of ceramide for the cellular effects of radiation.

The critical role of the acid sphingomyelinase in the cellular response to radiation is also very clearly evidenced in experiments on acid sphingomyelinase-expressing and -deficient oocytes (Morita et al, 2000). While oocytes of normal mice rapidly underwent apoptosis upon irradiation, those in acid sphingomyelinase-deficient mice survived.

Studies on normal and acid sphingomyelinase-deficient mice elaborated the cellular effects of radiation in detail. Whole body radiation of C57Bl/6 mice with doses less than 14 Gy resulted in predominant death of bone marrow cells and the mice died 12-14 days after radiation by deprivation of bone marrow cells (Paris et al, 2001). Accordingly, mice were rescued by bone marrow transplantation. An increase in dose above 15 Gy resulted

in severe alterations of the gastrointestinal tract with the development of a gastrointestinal syndrome (Paris et al, 2001).

The gastrointestinal syndrome is caused by depletion of villous and cryptic gland cells and characterized by a loss of the barrier and resorptive functions of the GI tract, which is very often lethal. Experiments from Paris et al (2001) evidenced that endothelial cells in small gastrointestinal vessels died by apoptosis as early as one hour. Apoptosis peaked in those cells already at 4 hours after 8 to 15 Gy irradiation, while apoptosis in epithelial cells in the crypts and villi occurred much later and was detected 8-10 hrs after irradiation. Endothelial cell apoptosis was radiation dose-dependent and the extent of apoptosis in endothelial cells correlated closely with the development of a gastrointestinal syndrome with massive endothelial apoptosis at 15 Gy radiation. In this correlation the borderline irradiation dose for death by delayed bone marrow insufficiency or immediate GI syndrome is crossed at 15 Gy.

In contrast, acid sphingomyelinase-deficient mice did not develop a gastrointestinal syndrome after whole body irradiation with 15 Gy and their endothelial cells did not undergo apoptosis. Moreover, intravenous injection of basic fibroblast growth factor that inhibits the acid sphingomyelinase protected normal mice from

development of a gastrointestinal syndrome even at doses as high as 17 Gy (Paris et al, 2001).

These studies employing a physiological stress response model indicated that irradiation primarily targets the acid sphingomyelinase in endothelial cells and proved that the acid sphingomyelinase is required for radiation-induced cell death *in vivo*.

Recent studies on a tumor model confirmed the notion that acid sphingomyelinase and ceramide play a central role for the induction of cell death (Garcia-Barros et al, 2003). Syngenic normal and acid sphingomyelinase-deficient mice were transplanted with the same tumors, i.e. B16F1 melanoma or MCA/129 fibrosarcoma. Therefore, any difference of tumor growth or in response to treatment must be caused by the differential expression of the acid sphingomyelinase in the tumor-bearing host animals. Radiation of tumors in normal mice resulted in a marked, more than 70% reduction of the tumor mass, while the same tumor was not affected by radiation in acid sphingomyelinase-deficient mice. The sensitivity of the tumor to radiation correlated with the induction of apoptosis in endothelial cells in tumor vessels of normal mice, while endothelial cells in tumor vessels of the acid sphingomyelinase-deficient mice failed to undergo apoptosis upon radiation. To illustrate the significance of acid sphingomyelinase in endothelial cells for the susceptibility of the tumor to radiation, Garcia-Barros et al. (2003) applied the finding that endothelial cells in tumor vessels are derived from two sources: The tumor requires the formation of novel blood vessels to extend a size of a few millimeters. Hence, tumor vessels are partly formed by proliferation of local endothelial cells and sprouting of preexisting vessels. However, a large proportion of endothelial cells in tumor vessels are derived from the bone marrow. Tumor cells release factors that mobilize and attract endothelial progenitor cells from the bone marrow that subsequently integrate in the newly formed tumor vessels. Transplantation of acid sphingomyelinase-deficient mice with normal bone marrow resulted in the incorporation of acid sphingomyelinase-positive endothelial progenitor cells into tumor vessels and restored sensitivity of the tumor to radiation. Vice versa, transplantation of normal mice with bone marrow cells derived from acid sphingomyelinase-deficient mice conferred resistance of the tumor to radiation. Finally, purification of endothelial cells from tumor vessels confirmed the *in vivo* data and showed that induction of cell death by radiation requires expression of the acid sphingomyelinase.

These data are not contradictory to the previous findings that tumors transplanted into SCID mice (Budach et al, 1993), which suffer from a defect in DNA-repair and are highly sensitive to radiation, did not show an increased radio-sensitivity. Since the acid sphingomyelinase has been shown to be activated by radiation in cellular membranes, the induction of apoptosis in endothelial cells by radiation via the acid sphingomyelinase might be independent of DNA damage and, thus, the sensitivity of the tumor might not be altered in SCID mice.

At present it is unknown how radiation-induced endothelial cell death in tumor blood vessels mediates

tumor reduction. Tumor cell death might be caused by tissue ischemia, leakage of humoral or cellular blood elements that might impact tumor cell viability and/or promotion of DNA double strand breaks within irradiated tumor cells.

Although the data evidence that endothelial cells are critically involved in the tumor's response to radiation, they do not exclude that the radiation response of other cells, e.g. tumor stroma cells (Schüler et al, 2003), is also determined by the acid sphingomyelinase. The integrity of tumor stroma cells has been shown to be required for tumor growth. If irradiation also affects these cells and alters the structural support provided by stroma cells to the tumor, the tumor cells might die. If the acid sphingomyelinase mediates the response of stroma cells to radiation, these cells might represent a second ceramide-sensitive population that is required for tumor growth.

In summary, these data indicate that bone marrow-derived cells, most likely endothelial precursor cells, are critical for the response of a tumor to radiation. The sensitivity or resistance of these cells is determined by expression and function of the acid sphingomyelinase. It is therefore of great interest to investigate, whether tumors are able to regulate the function of the acid sphingomyelinase in endothelial cells and to define the molecular basis of those mechanisms.

B. Ceramide and UV-A light

Although much less is known about the regulation of the acid sphingomyelinase and the role of ceramide in the mediation of UV-A effects, several data indicated that UV-A light rapidly induces activation of the acid sphingomyelinase, a release of ceramide and stimulation of c-Jun N-terminal kinase, while acid sphingomyelinase-deficient cells failed to respond to UV-A light (Zhang et al, 2001). Most important, expression of the acid sphingomyelinase in this setting was also required for the induction of apoptosis. Cells deficient for the acid sphingomyelinase were resistant to the induction of apoptosis by UV-A light (Zhang et al, 2001).

C. Ceramide and chemotherapy

Little is known about the role of the acid sphingomyelinase and ceramide in cytotoxic chemotherapy. It was shown that deficiency of the acid sphingomyelinase prevents induction of apoptosis in oocytes by the cytostatic drug doxorubicin, while acid sphingomyelinase-positive oocytes were sensitive to doxorubicin and died upon treatment (Morita et al, 2000). Likewise, incubation of oocytes with sphingosine 1-phosphate, which seems to antagonize many cellular effects of ceramide, prevented the induction of death in oocytes by doxorubicin (Morita et al, 2000; Paris et al, 2002). However, at present it is unknown, whether other cytostatic drugs also involve the acid sphingomyelinase pathway to trigger death in target cells and whether ceramide-enriched membrane platforms are important in this process.

D. Ceramide and development of tumors

Several data indicate that ceramide functions as a regulator of developmental cell death, at least in some cells. Acid sphingomyelinase-deficient mice display a defect in the developmental death of oocytes resulting in a marked increase in the number of oocytes in the ovarium at birth of the animals (Morita et al, 2000). Even at menopause the number of oocytes in acid sphingomyelinase-deficient mice still exceeds that in normal mice by approximately 10-fold. Therefore, it is interesting to note that recent data report a decrease of acid sphingomyelinase expression in some tumors. In particular, it was demonstrated that increasing malignancy of astrocytoma correlates inversely with acid sphingomyelinase expression, which was lowest in malignant glioma, i.e. astrocytoma grade IV (Riboni et al, 2002). Therefore, it is tempting to speculate that acid sphingomyelinase and ceramide balance pro-survival/pro-growth and apoptosis/death signals. A reduction in the expression of the acid sphingomyelinase might be part of the transition of a normal cell into a tumor cell. Whether this hypothesis can be applied *in vivo* has to be proven.

E. Ceramide and manipulations of the ceramide metabolism as novel treatment strategies of malignant tumors

Several drugs seem to mediate cell death via an activation of the acid sphingomyelinase, the release of ceramide, and the generation of ceramide-enriched membrane platforms.

It was demonstrated that many tumors are capable of reducing cellular ceramide concentrations by conversion of ceramide to glycosyl- or lactosylceramide (Lavie et al, 1996; Michael et al, 1997; Lucci et al, 1998; Liu et al, 2001). Inhibition of glucosyltransferases, which catalyze conversion of ceramide, increased the level of ceramide in the tumor cells and resulted in the induction of cell death. Furthermore, the inhibition of ceramide conversion amplified the effects of other cytostatic drugs on tumor cells and restored sensitivity of tumor cells to chemotherapy (Spinedi et al, 1998; Maurer et al, 2000). This demonstrates that tumor cells can actively decrease the cellular concentration of ceramide to prevent the accumulation of ceramide upon treatment with cytostatic drugs. However, whether an inhibition of ceramide consumption could improve treatment of tumors *in vivo* needs to be defined.

Finally, many tumor cells respond with cell death to ceramide analogues (Selzner et al, 2001). Short chain ceramides and ceramide analogues have been shown to regulate several molecules involved in apoptosis, including small G-proteins, protein kinase C, stress activated protein kinases, NF B, pro-apoptotic Bcl-2-like proteins, etc. Although these reagents are clearly pro-apoptotic *in vitro*, it remains to be determined whether they can be also utilized *in vivo* to treat tumors.

IV. Perspectives

The cellular ceramide concentration seems to regulate the grade of malignancy of tumors as well as the sensitivity of many tumor cells to treatment through radiation or chemotherapy. Therefore, many tumors seem to have developed strategies to reduce cellular ceramide, e.g. by downregulation of acid sphingomyelinase, glycosylation of ceramide or its de-acylation. Therefore, novel pharmacological or genetic strategies to restore or even increase the formation of ceramide or to block the consumption of this lipid in tumor cells or endothelial cells of tumor vessels may provide an opportunity to eliminate tumors by induction of apoptosis or by resensitization of the tumor to irradiation or chemotherapy.

Acknowledgments

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References

- Brown DA, and London E (1998) Structure and origin of ordered lipid domains in biological membranes. **J Membr Biol** 164, 103-114.
- Budach W, Taghian A, Freeman J, Gioioso D, and Suit HD (1993) Impact of stromal sensitivity on radiation response of tumors. **J Natl Cancer Inst** 85, 988-993.
- Cremesti A, Paris F, Grassmé H, Holler N, Tschopp J, Fuks Z, Gulbins E, and Kolesnick R (2001) Ceramide enables Fas to cap and kill. **J Biol Chem** 276, 23954-23961.
- De Maria R, Rippo MR, Schuchman EH, and Testi R. (1998) Acidic sphingomyelinase (ASM) is necessary for fas-induced GD3 ganglioside accumulation and efficient apoptosis of lymphoid cells. **J Exp Med** 187, 897-902.
- Fanzo JC, Lynch MP, Phee H, Hyer M, Cremesti A, Grassmé H, Norris JS, Coggeshall KM, Rueda BR, Pernis AB, Kolesnick R, and Gulbins E (2003) CD95 rapidly clusters in cells of diverse origins. **Cancer Biology and Therapy** 2, 392-395.
- Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, Fuks Z, and Kolesnick R (2003) Tumor response to radiotherapy regulated by endothelial cell apoptosis. **Science** 300, 1155-1159.
- Garcia-Ruiz C, Colell A, Mari M, Morales A, Calvo M, Enrich C, and Fernandez-Checa JC (2003) Defective TNF-alpha-mediated hepatocellular apoptosis and liver damage in acidic sphingomyelinase knockout mice. **J Clin Invest** 111, 197-208.
- Goni FM, and Alonso A (2002) Sphingomyelinases: enzymology and membrane activity. **FEBS Lett.** 531, 38-46.
- Grassmé H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, and Meyer TF (1997) Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells. **Cell** 91, 605-615.
- Grassmé H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, and Gulbins E (2001a) CD95 signaling via ceramide rich membrane rafts. **J Biol Chem** 276, 20589-20596.
- Grassmé H, Schwarz H, and Gulbins E (2001b) Surface ceramide mediates CD95 clustering. **Biochem Biophys Res Commun** 284, 1016-1030.
- Grassmé H, Jendrossek V, Bock J, Riehle A, and Gulbins E (2002) Ceramide-rich membrane rafts mediate CD40 clustering. **J Immunol** 168, 298-307.
- Grassmé H, Jendrossek V, Riehle A, von Kürthy G, Berger J,

- Schwarz H, Weller M, Kolesnick R, and Gulbins E (2003a) Host defense against *P. aeruginosa* requires ceramide-rich membrane rafts. **Nat Med** 9, 322-330.
- Grassmé H, Cremesti A, Kolesnick R, Gulbins E (2003b) Ceramide-mediated clustering is required for CD95-DISC formation. **Oncogene** 22, 5457-5470.
- Gulbins E, Bissonette R, Mahboubi A, Martin S, Nishioka W, Brunner T, Baier G, Baier-Bitterlich G, Byrd C, Lang F, Kolesnick R, Altman A, and Green D (1995) FAS-induced apoptosis is mediated via a ceramide-initiated Ras signaling pathway. **Immunity** 2, 341-351.
- Haimovitz-Friedman A, Kan CC, Ehleiter D, Persaud RS, McLoughlin M, Fuks Z, Kolesnick RN (1994) Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. **J Exp Med** 180, 525-535.
- Kirschnek S, Paris F, Weller M, Ferlinz K, Riehle A, Fuks Z, Kolesnick R and Gulbins E (2000) CD95-mediated apoptosis in vivo involves acid sphingomyelinase. **J Biol Chem** 275, 27316-27323.
- Kolesnick RN and Paley AE (1987) 1,2-Diacylglycerols, but not phorbol esters stimulate sphingomyelin hydrolysis in GH₃ pituitary cells. **J Biol Chem** 262, 9204-9210.
- Kolesnick RN, Goni FM, and Alonso A (2000) Compartmentalization of ceramide signaling. Physical foundations and biological effects. **J Cell Physiol** 184, 285-300.
- Lavie Y, Cao H, Bursten SL, Giuliano AE and Cabot MC (1996) Accumulation of glucosylceramides in multidrug-resistant cancer cells. **J Biol Chem** 271, 19530-19536.
- Li YQ, Chen P, Haimovitz-Friedman A, Reilly RM, and Wong CS (2003) Endothelial apoptosis initiates acute blood-brain barrier disruption after ionizing radiation. **Cancer Res** 63, 5950-5956.
- Liu YY, Han TY, Giuliano AE and Cabot MC (2001) Ceramide glycosylation potentiates cellular multidrug resistance. **FASEB J** 15, 785-791.
- Lucci A, Cho WI, Han TY, Giuliano AE, Morton DL and Cabot MC (1998) Glucosylceramide: a marker for multiple-drug resistant cancers. **Anticancer Res** 18, 475-480.
- Maurer BJ, Melton L, Billups C, Cabot MC and Reynolds CP (2000) Synergistic cytotoxicity in solid tumor cell lines between N-(4-hydroxyphenyl)retinamide and modulators of ceramide metabolism. **J Natl Cancer Inst** 92, 1897-1909.
- Michael JM, Lavin MF and Watters DJ (1997) Resistance to radiation-induced apoptosis in Burkitt's lymphoma cells is associated with defective ceramide signaling. **Cancer Res** 57, 3600-3605.
- Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, Fuks Z, Xie Z, Reed JC, Schuchman EH, Kolesnick RN and Tilly JL. (2000) Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. **Nat Med** 6, 1109-1114.
- Nurminen TA, Holopainen JM, Zhao H and Kinnunen PK (2002) Observation of topical catalysis by sphingomyelinase coupled to microspheres. **J Am Chem Soc** 124, 12129-12134.
- Paris F, Grassmé H, Cremesti A, Zager J, Fong Y, Haimovitz-Friedman A, Fuks Z, Gulbins E and Kolesnick R (2000) Natural ceramide reverses Fas resistance of acid sphingomyelinase (-/-) hepatocytes. **J Biol Chem** 276, 8297-8305.
- Paris F, Fuks Z, Kang A, Capodiecici P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C and Kolesnick R (2001) Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. **Science** 293, 293-297.
- Paris F, Perez GI, Fuks Z, Haimovitz-Friedman A, Nguyen H, Bose M, Ilagan A, Hunt PA, Morgan WF, Tilly JL and Kolesnick R (2002) Sphingosine 1-phosphate preserves fertility in irradiated female mice without propagating genomic damage in offspring. **Nat Med** 8, 1329.
- Pena LA, Fuks Z and Kolesnick RN (2000) Radiation-induced apoptosis of endothelial cells in the murine central nervous system: protection by fibroblast growth factor and sphingomyelinase deficiency. **Cancer Res** 60, 321-327.
- Riboni L, Campanella R, Bassi R, Villani R, Gaini SM, Martinelli-Boneschi F, Viani P, and Tettamanti G (2002) Ceramide levels are inversely associated with malignant progression of human glial tumors. **Glia** 39, 105-113.
- Santana P, Pena LA, Haimovitz-Friedman A, Martin S, Green D, McLoughlin M, Cordon-Cardo C, Schuchman EH, Fuks Z and Kolesnick R (1996) Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. **Cell** 86,189-199.
- Scheel-Toellner D, Wang K, Majeed S, Raza K, Curnow SJ, Salmon M, Lord JM (2002) The death-inducing signalling complex is recruited to lipid rafts in Fas-induced apoptosis. **Biochem Biophys Res Commun** 297, 876-879.
- Schüler T, Körnig S, and Blankenstein T (2003) Tumor rejection by modulation of tumor stromal fibroblasts. **J Exp Med** 198, 1487-1493.
- Schütze S, Potthoff K, Machleidt T, Berkovic D, Wiegmann K and Krönke M (1992) TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. **Cell** 71, 765-776.
- Selzner M, Bielawska A, Morse MA, Rudiger HA, Sindram D, Hannun YA and Clavien PA (2001) Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. **Cancer Res** 61, 1233-1240.
- Simons K, and Ikonen E (1997) Functional rafts in cell membranes. **Nature** 387, 569-572.
- Spinedi A, Bartolomeo SD and Piacentini M (1998) Apoptosis induced by N-hexanoylsphingosine in CHP-100 cells associates with accumulation of endogenous ceramide and is potentiated by inhibition of glucocerebroside synthesis. **Cell Death Differ** 5, 785-791.
- Zhang Y, Mattjus P, Schmid PC, Dong Z, Zhong S, Ma WY, Brown RE, Bode AM, Schmid HH and Dong Z (2001) Involvement of the acid sphingomyelinase pathway in UVA-induced apoptosis. **J Biol Chem** 276, 11775-11782.