Sphingolipids' Role in Radiotherapy for Prostate Cancer

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Contents

1 Radioresistance in Prostate Cancer: From Bedside to Bench 116
 1.1 Local Relapse After Radiation Therapy 116
 1.2 Human Prostate Cancer Cell Lines' Response to Radiation 117
 1.3 Radiation-Induced DNA Damage 117
 1.4 Radiation-Induced Apoptosis 118
2 Activation of the Ceramide Synthase Pathway in Response to Radiation 119
3 Modulation of Ceramide Synthase Activity in Prostate Cancer Cells 121
 3.1 PKCα Activation of Ceramide Synthase in Prostate Cancer Cells 121
 3.2 ATM Negatively Regulates Ceramide Synthase Activity 123
 3.3 PKCα Downregulation of ATM in Prostate Cancer In Vivo 124
 3.4 TNF-α and Fas Enhance Radiation-Induced Apoptosis in Prostate Cells 124
 3.5 Modulation of Prostate Cancer Cell Response via Acid Ceramidase Activity 125
4 Summary and Future Directions 126
References 126

Abstract There are several well-established mechanisms involved in radiation-induced cell death in mammalian cell systems. The p53-mediated apoptotic pathway is the most widely recognized mechanism (Lowe et al. Nature 362:847–849, 1993), although apoptosis has long been considered a less relevant mechanism of radiation-induced cell death (Steel, Acta Oncol 40:968–975, 2001; Brown and Wouters, Cancer Res 59:1391–1399, 1999; Olive and Durand, Int J Radiat Biol 71:695–707, 1997). We and others have recently focused instead on the emerging links between radiation, apoptosis, and ceramide and showed that ceramide is a

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sphingolipid-derived second messenger capable of initiating apoptotic cascades in response to various stress stimuli, including radiation.

Ceramide, the backbone of all sphingolipids, is synthesized by a family of ceramide synthases (CerS), each using acyl-CoAs of defined chain length for *N*-acylation of the sphingoid long-chain base. Six mammalian CerS homologs have been cloned that demonstrated high selectivity towards acyl-CoAs (Lahiri et al. FEBS Lett 581:5289–5294, 2007), and more recently, it was shown that their activity can be modulated by dimer formation (Mesicek et al. Cell Signal 22:1300–1307, 2010; Laviad et al. J Biol Chem 283:5677–5684, 2008).

This de novo ceramide synthesis has been observed in irradiated cells through a pathway normally suppressed by ataxia telangiectasia-mutated (ATM) protein, a key component of the cellular response to DNA double-strand breaks (Liao et al. J Biol Chem 274:17908–17917, 1999). ATM is not the sole factor known to affect apoptotic potential by modulating CerS activity. Recent work has also implicated protein kinase Cα (PKCα) as a potential CerS activator (Truman et al. Cancer Biol Ther 8:54–63, 2009).

In this review, we summarize involvement of CerS in sphingolipid-mediated apoptosis in irradiated human prostate cancer cells and discuss future directions in this field.

Keywords Prostate cancer • Radioresistance • Ceramide synthase • Ceramide • PKCα

1 Radioresistance in Prostate Cancer: From Bedside to Bench

1.1 Local Relapse After Radiation Therapy

Prostate cancer is the most commonly diagnosed non-skin cancer in men in the United States. It is estimated that 217,730 men were diagnosed with prostate cancer during 2010. About 32,050 died of the disease the same year (Jemal et al. 2010). Radiotherapy (RT) is a widely used modality for men with prostate cancer, but although radiation is capable of permanently eradicating localized prostate tumors, nearly 30 % of patients treated with potentially curative doses relapse at the sites of the irradiated tumors (Scardino and Wheeler 1988; Crook et al. 1995; Zelefsky et al. 1998). More recently, continued progress in RT delivery techniques has improved outcome through dose escalation. Nonetheless, although higher radiation dose levels were consistently associated with improved biochemical control outcomes and reduction in distant metastases, the biochemical relapse rate was still greater than 60 % at 5 years in high-risk patients (Zelefsky et al. 2008). These data indicate that prostate tumors vary in sensitivity to ionizing radiation. Furthermore, clinical data show that in patients who relapse locally after RT, initial treatment eliminates the great majority of the tumor cells, whereas a small fraction of tumor clonogens survive the lethal effects of radiation and eventually repopulate the irradiated site.

This observation indicates that there are variations in clonal sensitivity to the lethal effects of radiation even within a given tumor. Thus far, there have been neither a criteria for predicting the presence or prevalence of radiation-resistant tumor clones nor an effective approach to modulate the radiation response of human prostate tumor cells. Improved understanding of pathways of radiation-induced cell death and signaling systems that regulate these pathways may yield opportunities for pharmacological modulation of radiation resistance in prostate cancer.

1.2 Human Prostate Cancer Cell Lines' Response to Radiation

Cell lines derived from human prostate cancer are regarded as relatively resistant to both radiation-induced clonogenic death and apoptotic death (Royai et al. 1996). The best-characterized human prostate cell lines include the PC-3, DU-145, CWR22-Rv1, and LNCaP cells that were established from metastatic human tumor lesions (Stone et al. 1978; Kaighn et al. 1979; Horoszewicz et al. 1983). In general, these cell lines are among the most radioresistant human tumor cells, as assessed by the clonogenic assay (Wollin et al. 1989; Leith et al. 1993; Leith 1994; DeWeese et al. 1998). However, the dose-survival data do indicate distinct differences between these cell lines, as expressed by the Dq, D_0, SF-2, and the linear quadratic α and β exponents. There are also differences in the apoptotic response to radiation. Several studies reported lack of apoptosis in PC-3 cells up to 72 h after exposure to doses of 10–30 Gy (Algan et al. 1996; Li and Franklin 1998) and an incidence of 10–15 % apoptosis in DU-145 cells at 72 h after 10–12 Gy (Algan et al. 1996; Bowen et al. 1998). However, one study reported 40 % apoptosis in PC-3 cells at 72 h after 20 Gy (Kyprianou et al. 1997). This study is also the only study that has thus far reported apoptosis in LNCaP cells, occurring at a rate of 35 % at 72 h after exposure to 20 Gy (Kyprianou et al. 1997). Altogether, these observations indicate clone-specific sensitivities of human prostate tumor cells to radiation. The pleiotropic nature of death pathways induced by radiation suggests that radiation resistance is likely to be regulated by a variety of mechanisms, each of which is associated with a specific death pathway. Whether radiation resistance of human prostate tumor clones is associated with a single mechanism or a spectrum of mechanisms is unknown. These data also suggest that an approach to reduce radiation resistance clinically might require the use of combinations of chemical and biological modifiers to cover a spectrum of resistance mechanisms that may operate concomitantly in prostate cancer.

1.3 Radiation-Induced DNA Damage

It is well accepted that radiation-induced cell death stems from lethal DNA damage. When cells are irradiated, X-rays induce numerous DNA breaks. All break types do

not have the same biologic consequence as far as cell killing is concerned. Many DNA single-strand breaks are readily repaired using the opposite DNA strand as a template. Breaks in both strands, if well separated, are also readily repaired because they are handled independently. Breaks in both strands that are opposite or separated by only a few base pairs may lead to double-strand breaks (DSBs). There is increasing amount of evidence that DSBs rather than single-strand breaks lead to important biologic endpoints, including “mitotic catastrophe” and loss of replicative potential (Hall and Giaccia 2006a). Such lesions are produced in the DNA by direct interaction with X-rays or with reactive oxygen intermediates generated within the cell by the radiation. DNA DSBs, the most lethal form of ionizing radiation-induced damage, are repaired by nonhomologous end-joining (NHEJ) repair in the G1 phase of the cell cycle and homologous recombination (HR) repair pathway in the S/G2 phase of the cell cycle. Whereas most radiation-induced DNA DSBs are rapidly repaired by constitutively expressed DNA repair mechanisms, residual unrepaired or misrepaired breaks lead to genetic instability and to increased frequency of mutations and chromosomal aberrations. Lethal mutations or dysfunctional chromosomal aberrations eventually lead to either progeny cell death, usually after several mitotic cycles (also termed reproductive or postmitotic), or to p53-mediated apoptosis (Hall and Giaccia 2006b).

1.4 Radiation-Induced Apoptosis

Radiation-induced apoptosis has long been considered a less relevant mechanism in cell loss from normal tissues and tumors based on published data comparing apoptosis response and cell survival responses in tumor cells that have generally failed to find a causal relationship. Moreover, modulating apoptotic potential usually had little impact on cellular radiosensitivity (Steel 2001; Brown and Wouters 1999; Olive and Durand 1997; Kyprianou et al. 1997; Aldridge et al. 1995; Lock and Ross 1990). Apoptosis or programmed cell death is an intermitotic (interphase) inducible death pathway of sequential biochemical events that are constitutively expressed in an inactive form in most, if not all, mammalian cells. Also defined as a mechanism of cellular suicide, apoptosis occurs in response to a variety of physiological or environmental stresses impacting distinct cellular targets to initiate cell type-specific apoptotic signaling pathways. The various upstream signaling cascades converge downstream to activate a common final caspase-dependent effector mechanism eventually leading to activation of a calcium–magnesium-dependent endonuclease that cleaves the nuclear chromatin at selective inter-nucleosomal linker sites, thus dismantling the dying cell. Chromosomal fragmentation, cytoplasmic blebbing, and apoptotic bodies are consequently seen during apoptosis, which ultimately results in the condensation of the nucleus and shrinking of the cell. Apoptosis is characteristically different from cell

necrosis in morphology and biochemistry, and its end result is cell death without inflammation of the surrounding tissue. After a few decades and after quite a few debates, the case against apoptosis is no longer successfully defended. Today, we acknowledge apoptosis, or programmed cell death, not only as the process leading to disorders of normal tissues (Orrenius 1995; Fadeel et al. 1999; Reed 2002; Mullauer et al. 2001) but also as a form of death in response to both chemotherapy and radiation therapy for cancer, in particular for hormone-dependent cancers such as prostate cancer (Wu 1996; Olson and Kornbluth 2001; Ameisen 2002; Meyn et al. 2009). As a matter of fact, facilitation of apoptosis in vivo has been shown to effectively increase the number of apoptotic cells in tumors (Dubray et al. 1998; Jansen et al. 2000), and the detected early apoptotic response correlates well with subsequent outcome (Symmans et al. 2000; Meyn et al. 1995; Ellis et al. 1997). Proapoptotic antibodies, anti-CD-95 antibodies, are considered to be used as new therapeutic agents for tumor treatment. Moreover, the emerging links between radiation, apoptosis, and ceramide suggest that ceramide-mediated apoptosis following ionizing radiation might explain part of normal tissue and tumor cell death.

2 Activation of the Ceramide Synthase Pathway in Response to Radiation

We have previously shown that DNA DSBs induce ceramide generation via the de novo ceramide synthesis pathway involving activation of CerS enzyme in bovine aortic endothelial cells (BAEC) (Liao et al. 1999). Actually, a variety of ceramide species syntheses are catalyzed by six CerS enzyme isotypes (Table 1). CerS are integral membrane proteins of the endoplasmic reticulum. Once activated, these enzymes catalyze the condensation of sphinganine and fatty acyl-CoA to form dihydroceramide, which is rapidly oxidized to ceramide. Originally, we showed that daunorubicin stimulates CerS and generates ceramide resulting in apoptosis in P388 murine leukemia cells and U937 human monoblastic leukemia cells (Bose et al. 1995). An obligatory role for CerS was thus defined, since its natural specific inhibitor, Fumonisin B1 (FB1), blocked daunorubicin-induced ceramide elevation and apoptosis. This study was the first to demonstrate that CerS activity is regulated in eukaryotes and constituted definitive evidence for a requirement for ceramide elevation in the induction of apoptosis.

In collaboration with Anthony Futerman's group [Weizmann Institute of Science, Israel (Lahiri et al. 2007)], we demonstrated that despite the high selectivity towards acyl-CoAs, mammalian CerS have a very similar K_m value towards sphinganine, strengthening the notion that the main biochemical difference between CerS is in their specificity towards acyl-CoAs. In addition, in these studies, conditions for assaying CerS activity were optimized, and a K_m value of all six mammalian CerS towards sphinganine in the low μM range (2–5 μM) was demonstrated, which is

Table 1 Distinct ceramide species confer discrete and even opposing signaling endpoints including apoptosis and cell survival (*VLCFA*: *Very Long Chain Fatty Acid*)

CerS/LASS	Fatty acyl-CoA substrate chain length	Tissue specific expression patterns
CerS1	C18:0-fatty acyl-CoA	Nervous system
CerS2	C24:0 and C24:1 (VLCFA)	Broad tissue distribution
CerS3	C24:0 and C24:1 (VLCFA)	Testis and keratinocyte
CerS4	C18:0; C20:0	Broad tissue distribution
CerS5	C16:0	Broad tissue distribution
CerS6	C16:0	Broad tissue distribution

significantly lower than that suggested using a variety of other assays and tissue sources.

Subsequently, we reported that ionizing radiation also induces de novo synthesis of ceramide in HeLa cells resulting in apoptosis by specifically activating CerS5 and CerS6 (Mesicek et al. 2010). Overexpression of CerS2 resulted in partial protection from RT-induced apoptosis, whereas overexpression of CerS5 generated the apoptogenic ceramide species C_{16}-ceramide and increased apoptosis in these cells. Knockdown studies determined that CerS2 is responsible for all observable RT-induced C(24:0) CerS activity, and while CerS5 and CerS6 each confer approximately 50 % of the C(16:0) CerS baseline synthetic activity, both are required for RT-induced activity. Additionally, co-immunoprecipitation studies suggest that CerS2, 5, and 6 might exist as heterocomplexes in HeLa cells, providing further insight into the regulation of CerS enzymes. Moreover, CerS were shown to have additional subcellular localizations, such as perinuclear membranes and the mitochondria-associated membrane (MAM). The interplay between long-chain C_{16}-ceramide and very long-chain $C_{24:1}$- and C_{24}-ceramides has come into recent spotlight regarding their roles in maintaining cellular homeostasis. These data add to the growing body of evidence demonstrating interplay among the CerS isozymes in a stress stimulus-, cell type-, and subcellular compartment-specific manner.

CerS can be phosphorylated (Sridevi et al. 2009) and glycosylated (Mizutani et al. 2006), and it was recently demonstrated that CerS can form both homo- and heterodimers (Laviad et al. 2008) and that the activity of one member of a heterodimer depends upon, and can be modulated by, the activity of the other member. These studies suggest a rapid and reversible mechanism of regulating CerS activity by dimer formation.

CerS engagement in mammalian apoptosis in response to ionizing radiation, drugs, and cytokines (Truman et al. 2009; Garcia-Bermejo et al. 2002; Canman and Kastan 1995; Kastan et al. 1992) is a cell type-specific phenomena. The biological significance of CerS as a transducer of apoptosis has also been confirmed in vivo, as CerS transactivates disease pathogenesis in gastric ulcer, ischemia/reperfusion, and emphysema rodent models of human disease (Lowe et al. 1993; Spiegel et al. 1996; Haimovitz-Friedman et al. 1997; Lin et al. 2000; Mathias et al. 1998; Pena et al. 1997). In these reports, epithelial apoptosis occurred 18–24 h after radiation exposure or about 6–10 h after CerS activation and was inhibited by FB1.

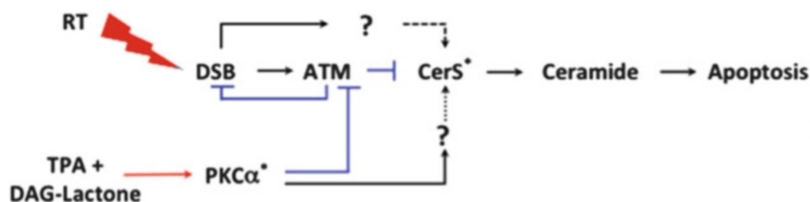

Fig. 1 Integration of radiation- and PKCα-induced and apoptosis (*Asterisk* denotes activated). TPA and DAG-Lactone simultaneously down-regulated ATM levels and enhance CerS activity in human prostate cancer cells *via* activation of PKCα. Radiation acts synergistically on CerS activation by inducing DNA DSBs, once the ATM inhibition is removed. This results in higher accumulation of ceramide levels in these cells and radiation-induced apoptosis both in vitro and in vivo [modified from Truman et al. (2009)]

3 Modulation of Ceramide Synthase Activity in Prostate Cancer Cells

There are several well-established mechanisms involved in radiation-induced apoptosis in mammalian cell systems. The p53-mediated pathway is the most widely recognized mechanism (Lowe et al. 1993). LNCaP and CWR22-Rv1 human prostate cell lines express wild-type p53 and exhibit extreme resistance to radiation-induced apoptosis. Yet, we found that these cells display high sensitivity to radiation-induced apoptosis both in vitro and in vivo when pretreated with TPA (12-*O*-tetradecanoylphorbol-13-acetate) or other PKC activators (Truman et al. 2009). Studies in our laboratory focus on ceramide-mediated apoptosis, a response to radiation that is distinct from the classical p53-mediated response. Our work indicates that the apoptotic pathway involved in LNCaP and CWR22-Rv1 radiosensitization is mediated by activation of CerS (Fig. 1), and not by other known pathways of ceramide-mediated apoptosis involving the activation of either acid sphingomyelinase (ASMase) or neutral sphingomyelinase (NSMase) (Garzotto et al. 1998, 1999).

3.1 PKCα Activation of Ceramide Synthase in Prostate Cancer Cells

TPA treatment of LNCaP cells activates PKCα and PKCδ to induce CerS activation (Garzotto et al. 1998) with rapid but progressive ceramide generation. This was followed by a delayed form of apoptosis that reached maximal levels at 48 h. Investigations into the mechanism of TPA-induced ceramide generation revealed that ASMase and NSMase activities were not enhanced. In contrast, TPA induced an increase in CerS activity that persisted for at least 16 h. Treatment with FB1 abrogated both TPA-induced ceramide production and

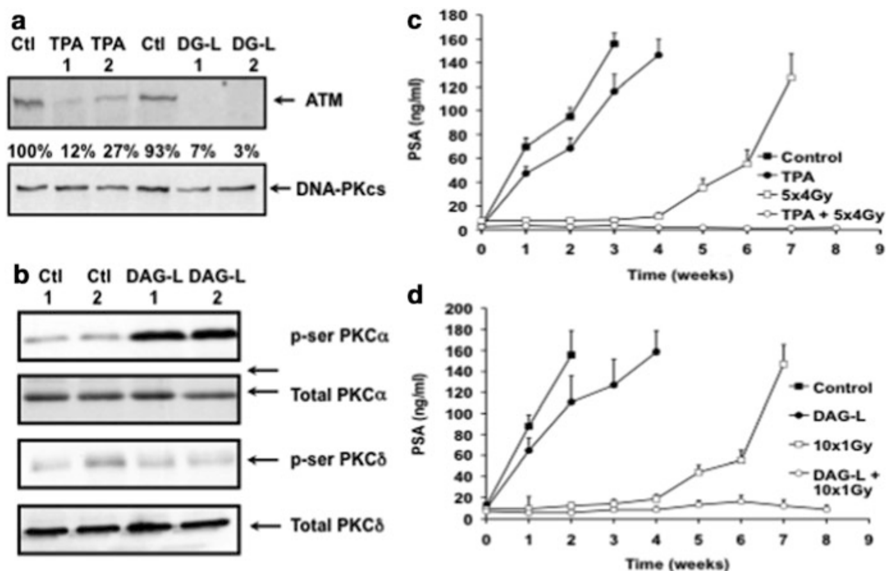

Fig. 2 Effect of PKCα stimulation on orthotopic prostate cancer model. (**a**) DAG-lactone was injected twice i.p. into orthotopically-implanted nude mice at 24 h intervals at a concentration of 12 mg/kg per day. Prostate tumors were removed 48 h after the first injection, homogenized and prepared for Western blot as described in the "Materials and Methods." The density of the bands was measured and is shown in comparison to the control. The results shown are representative of three separate experiments. (**b**) Western blots for prostate tumor samples were probed with either phospho-serine specific Abs to PKCα or PKCδ, and compared with total levels of PKCα or PKCδ as a control. The results are representative of two separate experiments. (**c**) PSA levels in TPA-treated mice. Mice were injected i.p. with 40 μg/kg TPA or DMSO solvent control for 5 days. Radiation-treated mice were locally irradiated with 4 Gy 16 h after each TPA injection for a total of 20 Gy. Control non-irradiated mice $n = 20$, radiated-treated control mice $n = 12$, TPA non-irradiated mice $n = 17$, and TPA-treated irradiated mice $n = 10$. (**d**) PSA levels in DAG-lactone treated mice. Mice were injected i.p. with 12 mg/kg per day of DAG-lactone or with DMSO solvent control for 10 days. Radiation-treated mice were locally irradiated at 1 Gy 16 h after each injection to a total of 10 Gy. Control non-irradiated mice $n = 14$, radiation-treated control mice $n = 14$, DAG-lactone treated mice $n = 13$, radiation-treated DAG-lactone mice $n = 12$. The values represent the mean ± standard errors (Truman et al. 2009)

apoptosis. Thus, CerS activation appears to be required for TPA-induced apoptosis in LNCaP cells (Garzotto et al. 1998).

Whereas LNCaP cells failed to respond to radiation with ceramide generation and apoptosis, pretreatment with TPA or diacylglycerol (DAG)-lactone, a potent and specific PKCα activator in LNCaP cells, converted this pattern, enabling radiation to signal CerS activation and apoptosis (Fig. 1) (Truman et al. 2005). The catalytic domain of activated PKCα is required for this event, since LNCaP cells transfected with a kinase-dead dominant-negative mutant of PKCα showed no increase in ceramide generation or in apoptosis levels, indicating that PKCα plays a role in CerS activation. Furthermore, treatment of nude mice with intravenous TPA

or DAG-lactone elicited specific PKCα activation leading to downregulation of ataxia telangiectasia-mutated (ATM) protein in prostate tumors in vivo and consequently enhanced radiation-induced tumor response in orthotopically implanted LNCaP cells (Fig. 2). This represents the first description of a signaling-based therapy designed to overcome one form of radiation resistance expressed preferentially in human prostate cancer cells (Truman et al. 2009).

3.2 ATM Negatively Regulates Ceramide Synthase Activity

By use of (Liao et al. 1999) metabolic incorporation of ^{125}I-labeled 5-iodo-2′deoxyuridine ([^{125}I] dURd), which produces DNA DSBs and external beam irradiation, de novo ceramide synthesis was initiated by posttranslational activation of CerS in BAEC. In the same study, it was shown that ATM negatively regulates CerS activity (Liao et al. 1999). Subsequently, it was shown that TPA decreases ATM protein levels in LNCaP and CWR22Rv1 cells, an event crucial for the induction of apoptosis and mediating radiosensitization of these cells. The alteration in ATM protein levels appeared to be due to inhibition of ATM transcription via decreased binding of transcription factor Sp1 to the ATM promoter. The additional TPA-mediated effector in downregulating ATM transcription remains unknown, nor is there information on the precise mechanism by which TPA affects Sp1 binding. The catalytic domain of activated PKCα is required for this event, since LNCaP cells transfected with a kinase-dead dominant-negative mutant of PKCα showed no decrease in ATM protein. The enabling of CerS-mediated apoptosis in both androgen-sensitive (LNCaP, CWR22-Rv1) and androgen-insensitive (PC-3, DU-145) prostate cancer cells via reduction of ATM protein levels indicates that the interaction between ATM kinase and CerS may represent a generic mode of regulation of radiation-induced death in these cells (Fig. 1). Whereas ATM kinase normally represses CerS activity, a reduction in ATM protein by TPA or antisense ATM oligonucleotide AS-ATM-ODN treatment attenuates CerS repression, enabling ceramide synthesis and a proapoptotic state. However, the experiments with AS-ATM-ODN indicated that ATM reduction by itself is not sufficient to induce CerS activation and that a second signal, such as that provided by radiation, is required. Consistent with this observation, TPA mimicked radiation in inducing apoptosis in AS-ATM-ODN-treated LNCaP cells.

ATM inactivation by AS-ATM-ODN conferred extreme radiation hypersensitivity, since the level of apoptosis attained in AS-ATM-ODN-treated LNCaP exposed to 2 Gy was already at 72 % of the maximum effect observed with 20 Gy (32 ± 0.73 %), both significantly higher than the 3.4 ± 0.2 % observed after 20 Gy exposure of ODN-untreated cells (Truman et al. 2005). The mechanism by which ATM kinase regulates CerS remains unknown. Whereas ATM inhibition has been shown to affect progression through the G2/M checkpoint in some cell types (Shiloh 2001), our studies indicate that deregulation G2/M is not the mechanism of prostate cancer cell radiosensitization. The significant apoptotic response

observed using clinically relevant doses of 1 Gy and 2 Gy in AS-ATM-ODN-treated cells suggests ATM as a potential molecular target for clinical application. Further development of AS reagents, siRNA, or small molecules aimed at ATM inactivation would appear warranted in the treatment of prostate cancer.

3.3 PKCα Downregulation of ATM in Prostate Cancer In Vivo

PKCα activation was shown to radiosensitize prostate cancer in vivo (Fig. 2). While fractionated radiation alone generated only a delayed tumor growth response in xenografts of human prostate tumors growing in nude mice, pretreatment with DAG-lactone followed by radiation resulted in permanent local tumor control. The detailed mechanistic pathway involved in this response remains unknown. We are currently trying to determine whether the radiation component of the apoptotic response in LNCaP cells is synergistic with the PKCα component or whether it promotes an autonomous hypersensitized response, from CerS, perhaps via direct activation of radiation-sensitive CerS homologs, whose activation is also enabled by PKCα-mediated ATM downregulation. Therefore, activation of PKCα regulates two main events necessary for the apoptotic response in these human prostate cells (1) ATM downregulation and (2) CerS activation. Since ATM is overexpressed in these cells, radiation is incapable of activating CerS unless a significant amount of ATM protein is downregulated (Fig. 1). In contrast to radiation-induced CerS activation, PKCα-mediated CerS activation does not depend on generation of DNA DSBs in these cells (Galvin and Haimovitz-Friedman, unpublished observations).

3.4 TNF-α and Fas Enhance Radiation-Induced Apoptosis in Prostate Cells

TNF-α was also shown to sensitize LNCaP prostate cancer cells to γ-radiation-induced apoptosis when added 24 h prior to radiation (Kimura et al. 1999). Simultaneous exposure of LNCaP cells to TNF-α and 8 Gy resulted in increased ceramide generation that correlated with a threefold increase in apoptotic cells within 72 h compared to TNF-α treatment alone. LNCaP cells could also be sensitized, although to a lesser degree, by the agonistic FAS antibody, CH-11 (Kimura and Gelmann 2000). In this study, TNF-α increased production of ceramide in LNCaP cells 48 h after exposure. Moreover, nontoxic levels of exogenous C_2-ceramide sensitized LNCaP cells to radiation similarly to TNF-α, suggesting that increased intracellular ceramide could explain the mechanism by which LNCaP cells were sensitized to radiation and even to chemotherapy. Further studies proved that TNF-α induced enhanced activation of the intrinsic

apoptotic pathway and enhanced cell death, with or without γ-irradiation, yet CerS activity was not reported. Both TNF-α and γ-irradiation elevated levels of endogenous ceramide and activated the intrinsic cell death pathway in a synergistic fashion (Kimura et al. 1999, 2003).

Moreover, it was shown in LNCaP cells that androgen inhibits apoptosis induced by both TNF-α and by CD95 activation with or without concomitant irradiation. This was thought to be mediated by androgen blockade of caspase activation in both intrinsic and extrinsic cell death pathways, but whether SMase is involved in LNCaP cell apoptosis remains to be proven (Kimura et al. 2003). In addition, TNF-α and radiation induced a significant increase in sphingosine levels and markedly reduced sphingosine-1-phosphate (S1P).The increase in sphingosine levels either by exogenous sphingosine or by treatment with the sphingosine kinase (SphK) inhibitor induced apoptosis and also radiosensitized LNCaP cells in this study.

Increasing amount of data now suggest that the relative levels of proapoptotic sphingolipid metabolites, such as certain ceramide species and sphingosine, and the levels of the antiapoptotic sphingolipid metabolites, such as S1P, might play a role in determining the radiosensitivity of prostate cancer cells (Nava et al. 2000). In fact, SphK1 and S1P receptors are highly expressed in chemotherapy-resistant prostate cancer PC3 cells and are upregulated by anticancer drug camptothecin (Akao et al. 2006). Pchejetsky et al. (2008) showed that selective pharmacologic inhibition of SphK1 triggers apoptosis in LNCaP and PC3 cells, an effect reversed by SphK1 enforced expression. Nevertheless, further investigations are necessary to clarify the role of SphK1 and S1P in apoptosis in prostate cells.

3.5 Modulation of Prostate Cancer Cell Response via Acid Ceramidase Activity

Acid ceramidase (AC) converts ceramide into sphingosine and was found to be overexpressed in 60 % of primary prostate cancer tissues (Liu et al. 2009; Norris et al. 2006; Seelan et al. 2000). Conversely, upregulation of AC in prostate cancer cells conferred resistance to both chemo- and radiotherapy (Saad et al. 2007; Mahdy et al. 2009). Along the same line, autophagy was increased in prostate cancer cells overexpressing AC, thereby enhancing resistance to C_6-ceramide. This resistance was overcome via modulation of radiation effect by using AC inhibitors (Liu et al. 2009). Interestingly, in another hormone-regulated cancer, of the breast, C_{6-}ceramide and targeted inhibition of AC were also shown to induce synergistic decreases in the cancer cell growth (Flowers et al. 2012).

Resveratrol (3,5,4′-*trans*-trihydroxystilbene), a natural product from grapes and present in red wine, was shown to be synergistic with radiation in androgen-independent and otherwise radioresistant DU145 human prostate cancer cell line by promoting de novo ceramide biosynthesis in these cells, but no mechanism was suggested (Scarlatti et al. 2007).

4 Summary and Future Directions

In conclusion, both DNA DSB-induced damage and non-DNA DSB-induced damage contribute to cell killing of human prostate cancer cells after exposure to ionizing irradiation. The relative contribution from each mode of cell death may differ with dose and from one cell type to another, relative to their inherent and inducible capacities to overcome each of these types of lethal radiation damage and according to their microenvironment. Utilization of the SMase pathway for induction of apoptosis in response to cell death receptor signals and ionizing radiation has now been demonstrated in a large number of mammalian cells. An alternative mechanism to SMase-mediated generation of ceramide in response to stress is a pathway that involves de novo synthesis of ceramide, via activation of CerS. CerS activation was shown to be of particular importance in a variety of human prostate cancer cell lines (LNCaP, CWR22-Rv1, PC3, and DU145) that under normal conditions exhibit extreme resistance to radiation-induced apoptosis. Yet we found that these cells display high sensitivity to radiation-induced apoptosis both in vitro and in vivo when pretreated with specific PKCα activators. We have also demonstrated that PKCα activation suppresses ATM, derepressing CerS activity, thus enabling generation of apoptogenic ceramide.

Our in vivo data using the prostate orthotopic model suggest that development of radiation response modulators for human prostate cancer affecting the ATM/CerS pathway is essential to overcome radiation resistance in this cancer. Recently, a selective tissue and subcellular distribution of the six mammalian CerS isoforms, combined with distinct fatty acyl chain length substrate preferences, has been described (Table 1) (Mesicek et al. 2010). This implicates differential functions of specific ceramide species in cellular signaling in a stress stimulus-, cell type-, and subcellular compartment-specific manner. Understanding the contribution of the different CerSs homologs and their mode of activation within the different prostate cancer cell types may provide new therapeutic molecular targets to manipulate the radiation response of these cells. These studies may pave the way for future studies looking at DNA-damage-inducing chemotherapeutic drugs and radiation to be used in combination with specific PKCα activators and molecular targets within the sphingolipids metabolic pathways to combat metastatic prostate cancer. Using DAG-lactone as a radiosensitizer via activation of PKCα is currently being considered as a therapeutic approach for prostate cancer at our center.

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