MINI-REVIEW

Ionizing Radiations Induce Apoptosis in TRAIL Resistant Cancer Cells: *in vivo* and *in vitro* Analysis

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Abstract

Increasingly it is being realized that despite considerable advancements in therapeutic interventions related to treatment of cancer, satisfactory results are still difficult to achieve. Rapidly accumulating evidence has started to shed light on the fact that cancer cells escape from death via constitutive activation of pro-survival signaling cascades. Cell biology and genetics have extensively enhanced our current understanding of the molecular mechanisms that underlie loss of apoptosis in cancer cells. This review is focused on ionizing radiation mediated restoration of TRAIL mediated apoptosis as evidenced by cell culture and animal model studies. Moreover, we also bring to the limelight radiation induced expression of miRNAs and how miRNAs further control response of cancer cells to radiation.

Keywords: Ionizing radiation - TRAIL - apoptosis

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Introduction

TRAIL mediated signaling has gained tremendous appreciation because of its ability to selectively induce apoptosis in cancer cells and leaving non-cancer cells intact. Data obtained through increasingly sophisticated laboratory methodologies, is deepening our understanding about intracellular signaling modulators of TRAIL. It is now well established that there are wide ranging regulators of TRAIL mediated signaling cascade. However there was a paradigm shift in the research trends when it was reported continuously by various research groups that different cancer cells were resistant to TRAIL induced apoptosis. *In vitro* analysis and gene silencing strategies helped in expanding the signaling landscape. TRAIL induced apoptosis in cancer cells via Death receptors DR4 and DR5. There are two well studied pathways through which TRAIL induced apoptosis including extrinsic pathway and intrinsic pathway. Research over the years has provided exciting pieces of evidence which describe various steps of TRAIL mediated signaling. Binding of TRAIL to TRAIL receptor (DR4 or DR5) resulted in receptor oligomerization. Later, Death receptor, FADD and Pro-caspase-8 together form Death Inducing Signaling Complex (DISC). However, cFLIP is an anti-apoptotic protein and has two death-effector domains (DEDs). cFLIP negatively regulates TRAIL induced signaling by interfering with the activation of caspase-8. Caspase-8 activates its downstream effector caspase-3. Intrinsic pathway is activated when Bid (apoptotic protein) undergoes cleavage and enters into mitochondria to facilitate release of pro-apoptotic proteins including cytochrome c, SMAC/DIABLO and Omi/Htra. Cytochrome c, procaspase-9 and apoptotic protease activating factor mediated activation of caspase-9 resulted in downstream effector activation. Pathway is shown in Figure 1.

It is becoming progressively more understandable that TRAIL resistant cancer cells can be re-sensitized

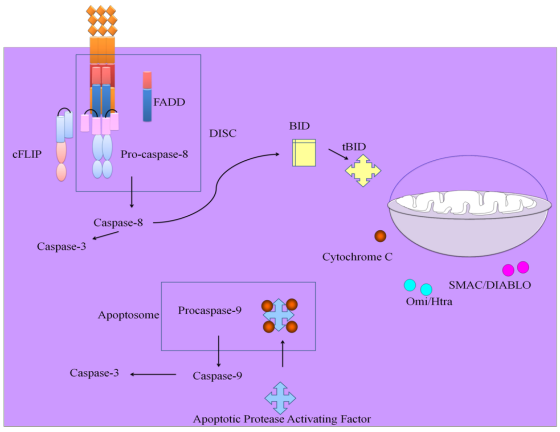

Figure 1. Shows TRAIL Receptor Mediated Transduction of Signals Intracellularly

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to TRAIL using different approaches. Consistent with this approach, IR treated glioma cells displayed a higher apoptotic rate. Experimental data verified the fact that IR and TRAIL induced apoptosis via functionalization of intrinsic pathway (Nagane et al., 2007). In line with similar approach, another contemporary study suggested that p53-mutant leukemic cells pre-exposed to IR displayed significantly higher DISC formation and consequent activation of Caspase-8. Gene silencing strategies were used to verify if apoptosis was induced via intrinsic pathway. Targeted inhibition of Bax and Bak and cancer cells reconstituted with dominant negative Caspase-9, irrespectively demonstrated apoptosis upon pretreatment with IR, thus underscoring the fact that extrinsic pathway was involved (Verbrugge et al., 2008). It has previously been convincingly revealed that combinatorial treatment of TRAIL with irradiation strongly induced apoptosis in breast cancer cells and colorectal cancer cells. It was noted that there was an upregulated expression of DR5 in irradiated cancer cells (Marini et al., 2005).

Confluence of information highlighted the fact that xenografting gastric adenocarcinoma MKN45 and MKN28 cells in SCID mice developed tumor. Interestingly, it was shown that X irradiation treated xenografted mice displayed a remarkably reduced tumor growth (Takahashi et al., 2008). It has also been noted in TRAIL resistant T-lymphoblastic leukemia cells that pre-exposure of cells to IR restored sensitivity to TRAIL in cancer cells (Rezacova et al., 2008). It is appropriate to mention that tumor xenografted nude mice upon irradiated for 5 days at 5x3Gy displayed marked regression of tumor growth in nude mice (Marini et al., 2009). It is intriguing to note that IR treated osteosarcoma cells displayed substantially enhanced transcription of DR4 and DR5. Moreover, there was a decline in cellular levels of c-FLIP and XIAP (Hori et al., 2010).

IR Mediated Control of miRNAs

It is surprising to note that irradiated cervical cancer cells showed an upregulated expression of pro-apoptotic miR-193a-3p. Additionally it was noted that miR-193a-3p negatively regulated anti-apoptotic gene Mcl-1. Cancer cells reconstituted with Mcl-1 represented considerably reduced IR induced apoptosis (Kwon et al., 2013). In pancreatic cancer cells it has been shown that IR reduced expression of miR-99b that resulted in restoration of mTOR expression. mTOR expression has phenotypic effects and targeted inhibition of mTOR is necessary to improve IR induced apoptosis in cancer cells (Wei et al., 2013) shown in Figure 2.

In human lung cancer cell lines it has been shown that miR-210 is involved in inducing resistance against radiation (Grosso et al., 2013). Targeting of miR-210 was tested in nude mice xenografted with hepatoma cells. Results indicated that knock down of miR-210 dramatically reduced tumor progression in xenografted mice (Yang et al., 2013). miR-375 over-expressing gastric cancer cells are also resistant to radiations, like shown in Figure 2. *In vitro* analysis revealed that miR-375 negatively regulated p53 and cancer cells reconstructed with p53 overcome

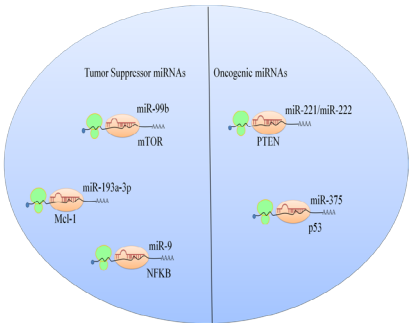

Figure 2. Provides an Overview of Tumor Suppressor MiRNAs and Oncogenic MiRNAs. mTOR and NFKB are well studied modulators which are reported to be involved in induction of resistance to apoptosis. miRNA studies have provided convincing evidence that loss of tumor suppressor miRNAs results in restoration of expression of oncogenes. MiR-9, miR-193a-3p and miR-99b negatively regulate NFKB, Mcl-1 and mTOR. PTEN is a negative regulator of AKT and it is frequently inactivated in mutationally activated Akt containing cancer cells. P53 is also quantitatively repressed by miR-375

miR-375 induced resistance to radiations in cancer cells (Liu et al., 2013). It has been shown that pro-survival factor, NFKB is negatively regulated by miR-9 in cancer cells and enhance radiosensitivity. Cancer cells usually have down-regulated miR-9 however cells reconstructed with miR-9 considerably improved radiosensitivity via negative regulation of NFKB (Arora et al., 2011). More importantly, PTEN a phosphatase reported to be involved in inhibiting Akt is under-expressed in cancer cells. miRNA studies have started to expand our understanding about the fact that PTEN is negatively regulated by miR-221/miR-222. Therefore targeted inhibition of miR-221/miR-222 is effective in enhancing radio-sensitivity (Zhang et al., 2011).

Breast cancer cells are mechanistically resistant to radiation and it is noteworthy that enforced expression of miR-302 in cancer cells resulted in an improved apoptotic response (Liang et al., 2013).

Conclusion

We have attempted to provide an overview of IR induced apoptotic response in resistant cancer cells. Substantial fraction of information has been added into the ever-expanding list of synthetic and natural agents which can be useful in restoring apoptosis in resistant cancer cells. Cancer is a multifaceted disease and it is now evident that multipronged approach is more effective. In-vivo study using prostate cancer mouse model, Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) has shown that low dose or high dose radiation did not regulate progression or regression of cancer in the model (Lawrence et al., 2013).

However there are some aspects which need detailed investigation. Although, IR effectively induced apoptosis in radio-sensitive cancer cells however, in certain situations cancer cells use DNA damage repair machinery to repair damaged DNA that might add another layer of complexity. TMPRSS2-ERG in prostate cancer cells

and BCR-ABL in leukemic cells are some of the well studied examples and fusion positive cancer cells rewire the signaling cascades. On a similar note there are wide ranging negative regulators in cancer cells which contribute to radio-resistant phenotype. Well appreciated example is PI3K/mTOR signaling axis which is activated in irradiated cancer cells. Cancer cells treated with NVP-BEZ235 and NVP-BGT226, dual PI3K/mTOR inhibitors displayed sensitivity to ionizing radiation (Fokas et al., 2012; Zhu et al., 2013). As discussed in miRNA section that IR treated cancer cells had over-expressed mTOR via suppression of its negative regulator, miR-99b. All these aspects are insufficiently studied in different cancers and a deeper understanding of IR induced transcriptional and translational changes in cancer cells will be helpful in getting a step closer to personalized medicine.

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