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REVIEW ARTICLE

Genetic Extermination of Cancer Cells

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ABSTRACT

Context: Targeted cancer therapy has lately been the preferred cancer treatment modality. It is principally based on silencing or downregulating of specific genes responsible for cancer.

Extensive exploration in this field has revealed the fact that CD95/CD95 ligand (CD95L), apart from their apoptotic role in killing cancer cells, also plays a non-apoptotic role in tumor advancement. Scientists have discovered that by eliminating CD95/CD95L, there is considerable decrease in tumor growth and invasiveness.

Objective: Elimination of CD95/CD95L may offer a novel approach to targeted cancer therapy by suppressing the multiplication of tumor cells and thereby ensuring their death.

Materials and methods: Data were obtained and analyzed from electronic database searches of relevant published literatures from PubMed and Google Scholar.

Conclusion: Targeting the multiple tumor-promoting activity of CD95/CD95L is a potent treatment modality that can better the prognosis and increase the survival rate of cancer patients.

Keywords: CD95, CD95 ligand, Death domain, Death-induced CD95/CD95 ligand elimination, Death-induced survival gene elimination, Messenger ribonucleic acid, Ribonucleic acid interference, Short hairpin ribonucleic acid, Small interfering ribonucleic acid.

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INTRODUCTION

Cancer has turned out to be a major public health problem throughout the world. Focus has shifted toward formulating targeted therapies for treatment of cancer. Every cellular change, including proliferation, differentiation, and tumorigenesis, occurs through the activation or inactivation of the related molecular signaling pathways. The important signaling molecules can be overexpressed or underexpressed. Targeted therapy in oncology involves administration of biological reagents which inhibit the biological pathways as well as target oncogenes usually overexpressed in cancer. This treatment modality mainly uses the principle of gene silencing, whereby it downregulates the expression of a specific gene which is often the culprit.[1,3,5]

However, such targeted therapy has been futile to deliver a complete cure for the majority of cancer patients with advanced cancer. Often, patients develop resistance to the treatment because of mutations in the targeted gene. The necessity now is to formulate a radically different therapeutic modality which, rather than targeting individual genes, can identify and disrupt networks of genes. Toward this end, scientists have discovered specific molecules called assassin ribonucleic acid (RNA) molecules which can trigger a mechanism wherein cancer cells are prevented from becoming resistant and also eliminate multiple survival genes. Surprisingly, researchers also found that these special RNA molecule sequences can be found all through our genome, inserted in multiple genes. These silent RNA suicide molecules trigger a mechanism called "death by induced survival gene elimination," which, if activated, will eliminate cancer cells with no injurious consequence on normal cells.[1,2,16]

This review discusses the mechanistic facets of RNA interference, off-target effect (OTE), biological role of CD95 and CD95L in immune homeostasis, role of CD95/CD95L in tumor progression, death-induced CD95/CD95L elimination (DICE), death-induced survival gene elimination (DISE), and its beneficial aspects in cancer therapy.

RNA INTERFERENCE AND OFF-TARGET EFFECT

Genetic make-up of an individual is warehoused in molecules of deoxyribonucleic acid (DNA). When the body is in need of a particular protein, these data are transferred to chemically similar molecules called messenger RNAs (mRNAs) by the process of transcription. These mRNAs undergo translation to build proteins.[3,4]

Apart from the mRNAs, some of the regulatory noncoding RNAs like micro RNA (miRNA), small interfering RNA (siRNA), etc., take part in controlling the upregulation or downregulation of specific gene encoding a specific protein. They mainly do this by a process called

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as RNA interference wherein miRNA/siRNA/shRNA endogenously or exogenously produced will specifically block the target mRNA and thus hinder the synthesis of protein. This is the basic mechanism used by scientists to target a specific gene in the treatment of cancer. CD95 and CD95L are two proteins which have been broadly analyzed in RNA interference (RNAi) experiments since they are involved in tumor progression, and targeting them through RNAi had shown reduction in tumor growth. Though RNAi experiments are aimed to be specific, at times, they can unintentionally attack other nontarget messenger RNAs. This phenomenon is called as off target effect. The siRNA used to target CD95/CD95L by RNAi had targeted other survival genes essential for the cancer cells. Hence, scientists have hypothesized that RNAi OTE can be used to kill cancer cells.[1,3,5]

CD95/CD95L COMPLEX IN IMMUNE SYSTEM

CD95 [Fas/APO-1/tumor necrosis factor receptor superfamily (TNFRSF)6] is a cell surface protein that forms a part of tumor necrosis factor receptor super family. Members of this family induce apoptosis when it binds to its ligands, or interacts with agonistic antibodies. They are capable of doing so because of the presence of a cytoplasmic death domain (DD) (conserved stretch of 80 amino acids in its cytoplasmic tail). Possession of the DD makes a subdivision of the TNFRSF known as death receptors.[6,7] Together with CD95, other death receptors include TNF-R1, TNF-related apoptosis-inducing ligand (TRAIL)-R1 (DR4), TRAIL-R2(APO-2/TRICK/DR5/KILLER), DR3 (TRAMP/APO-3), and DR6. They are activated by their corresponding ligands like TNF, TRAIL, and TNF-like ligand 1A (TL1A). Ligand for DR6 is not known. Thus, CD95 (APO-1/Fas) is involved in immune surveillance by killing cancer cells and virus-infected cells through initiation of apoptosis. They are also related to a myriad of pro-survival and migratory signaling pathways in tumor.[7-9]

EXPRESSION OF CD95L

The CD95L is chiefly expressed in activated T lymphocytes, natural killer cells, surface of neurons, corneal epithelia, and endothelia. Recently, studies have shown that the ligand is also found on the surface of endothelial cells lining blood vessels feeding cancer tissues. The transmembrane form of CD95L is a powerful inducer of cell death. The m-CD95L in cancer cells can be sliced by different metalloproteases to release s-CD95L (soluble CD95L). It cannot only eliminate tumor and virus-infected cells but also execute T-cells by a process called activation-induced cell death.[9-11]

CD95/CD95L-INDUCED APOPTOSIS

Apoptosis in a cell occurs by two mechanisms: intrinsic and extrinsic pathways. Extrinsic pathway occurs by activation of death receptors, for instance, Fas, which have an intracellular death domain. This results in the formation of a death-inducing signaling complex (DISC) in which the initiator, caspase-8, is activated by its adaptor Fas-associated death domain (FADD). This results in activation of the caspase cascade of which the most important is the activation of caspase 3. When caspase-3 is activated, it chops many vital cellular proteins accountable for the characteristic biochemical and morphological hallmarks of apoptosis.[11,12]

NON-APOPTOTIC ROLE OF CD95

CD95 has various non-apoptotic actions. CD95 is involved in liver regeneration after partial hepatectomy, stimulation of renal tubular epithelial cell movement by an integrin-dependent mechanism, and providing a mitotic signal in dormant hepatic stellate cells through activation of epidermal growth factor receptor.[13,14] CD95 is also imperative for neurite outgrowth. CD95 is considered to be a powerful activator of neurogenesis in both normal and injured brains where it enhances neuronal stem cells. CD95 and CD95L have tumor-promoting action also. Consistent with this hypothesis are data establishing roles of CD95 in stimulating cancer cell migration, growth, and epithelial–mesenchymal transition.[1,2,15,16]

BIOLOGICAL ROLE OF CD95 AND CD95L IN CANCER

In the perspective of cancer, CD95 has revealed its tumor-promoting activities and this concept is gaining traction in the field of research. Despite the fact that CD95/CD95L is a major apoptotic system used by cytotoxic T lymphocytes to eliminate neoplastically transformed cells, most of the cancer cells are resistant to CD95-mediated apoptosis.[17-19] Cancer cells have manifold ways of becoming resistant to apoptosis mediated by CD95. In a nutshell, these cells procure survival advantage in the course of tumor progression by reducing its sensitivity to CD95-induced apoptosis by the following mechanisms.[20,22]

- Downregulating expression of CD95 protein.
- Blocking of receptor site by soluble form of CD95L.
- Blocking interaction of CD95 with CD95L by soluble decoy receptor 3.
- CD95 apoptotic signal can be inhibited at the level of the DISC by increasing expression of cFLIP (cellular FLICE inhibitory protein), which in turn inhibits the interactions of caspase-8 and -10 with DISC, or by reducing the expression of FADD or caspase-8.

- Deregulating the expression of proteins belonging to Bcl-2 family or inhibiting the apoptosis proteins, thereby favoring tumor survival.

THE TUMOR-PROMOTING ACTIVITIES OF CD95

CD95 expressions are advantageous to cancer cells for the reason that it can transform cancer cells into a more de-differentiated state. CD95L is usually expressed in two forms, a membrane-bound form and a soluble form, which is created by slicing of mCD95L by metalloproteinases. mCD95L is indispensable for apoptosis induction, whereas sCD95L has non-apoptotic activities and possesses principal tumor-promoting activity.[16,17,19,21] The m-CD95L when interacting with CD95 leads to the formation of DISC, whereas s-CD95L interaction leads to the formation of a molecular complex without FADD and caspase 8, which in turn recruits and activates the src kinase c-yes by means of generation of nicotinamide adenine dinucleotide phosphate oxidase 3 and ROS. This complex is designated as motility-inducing signaling complex and are assumed to be part of micrometastatic dissemination of cancer cells. Large quantities of s-CD95L can be detected in cancer patient serum. CD95 alters apoptosis-resistant differentiated cells or noncancer stem cells (non-CSCs) into cancer stem cells (CSCs) and sustain CSCs in the tumor microenvironment. Apparently CD95 is considered to be a driver of epithelial to mesenchymal transition in cancer cells. CD95 is involved in activation of NF-kB and the three mitogen-activated protein kinases, JNK1/2, p38, and Erk1/2. In addition, studies have demonstrated that CD95-mediated invasiveness in cancer cell lines requires activation of NF-kB and ERK. It is now generally recognized that once cancer cells attain resistance to CD95-mediated apoptosis, additional stimulation of CD95 is tumorigenic.[1,2,11,13] Other studies have reported that CD95 facilitates invasion by the use of the Src/PI3K/GSK3$/matrix metalloproteinase pathway.

Hence, it has become understandable that CD95/CD95L has various tumor-promoting activities. CD95 signaling stimulates cell growth that hastens motility and invasiveness of tumor cells, and promotes cancer stemness.[1,2,16,18]

Since CD95 contributes to multiplication of tumor cells, studies have lately affirmed that the elimination of CD95 or CD95L executes cancer cells (*in vitro* and *in vivo*) in a process termed as DICE.

DEATH INDUCED BY CD95/CD95L ELIMINATION AND DEATH INDUCED BY SURVIVAL GENE ELIMINATION

CD95L is produced by cancer cells to provide low-level survival signaling through mutations occurring in CD95 gene. When CD95/CD95L were downregulated by siRNAs or short hairpin RNAs (shRNAs), there was evident dropping in the rate of tumor growth and if the knockdown was constant for a longer period of time, the cancer cells were dead. According to prior studies, knockdown of either CD95 and/or CD95L in about 15 cancer cell lines caused extensive induction of cell death in every cell line. This phenomenon is termed as DICE.[1,2,11,13] When siRNAs/shRNAs were used to target CD95/CD95L, scientists have noticed induction of a peculiar pattern of cell death charecterized by synchronized activation of multiple cell death pathway slaughtering transformed and cancer stem cells. Extensive experimental investigation on the same led scientists to conclude that this form of death resulted from OTE by siRNAs/shRNAs when they were used to knock down CD95/CD95L. Thus, cells were dying due to the silencing of multiple critical survival genes which in turn activated multiple cell death pathways. They therefore named this sort of cell death as DISE.[1,2,14,18] Thus, DISE is a creation of unique form of OTE. In addition, this unique form of cell death cannot be repressed by conventional cell death or signaling pathway inhibitors or by downregulation of any single gene.

The DISE is characterized by swelling of the cells, appearance of large dilated vacuoles, signs of autophagy, production of reactive oxygen species, and DNA double-strand breaks. This is in turn followed by reduction in cell viability, activation of caspase 2, mitochondrial outer membrane permeabilization, and eventually cell death. In a nutshell, DISE represents multiple cell death pathway terminating in a necrotic pattern of mitotic catastrophe with signs of apoptosis, autophagy, and senescence. Mitotic catastrophe is a form of cell death taking place in the course of mitosis, in accordance with DNA damage or deranged spindle formation combined with debilitation of different checkpoint mechanisms that would generally halt progression into mitosis. Autophagy is an exceptionally structured molecular process in which misfolded proteins and organelles are carried to lysosomes for their degradation. As per the studies, DISE was not inhibited by any of the 1,200 tested drugs or by knocking down of any single gene in a genome-wide shRNA screen, which proposes that it is a dynamic cell death machinery difficult to block.

The DISE is yet anonymous in cancer surveillance mechanism. Induction of DICE pooled with induction of canonical CD95-mediated apoptosis results in almost complete eradication of cancer cells. The DISE is not a mechanism which prevents tumor development; instead, it prevents survival of existing tumor cells that lose CD95 functionality and therefore could not be eliminated by immune cells.[13,14,16]

CONCLUSION

In this review, a brief analysis of the role of CD95/CD95L in cancer and how a newer treatment modality can be implemented by targeting the same has been discussed. Inducing DISE in cancer cells by targeting CD95/CD95L could be an innovative and powerful means to exterminate cancer. It is thus likely to be promising that CD95 or CD95L could be safely targeted for therapeutic purposes, as it blocks all the tumorigenic properties and activates multiple death pathways which kill cancer cells. Scientists have recently identified other genes in genome which contain DISE-inducing shRNA. Therefore, it is likely that when cells are exposed to genotoxic or oncogenic stress, they produce abundant small RNAs that can be taken up by RISC and, in combination, execute DISE.[22-25]

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