

Recent Patents Relating to Tumor Suppressor Genes

Jason J. Derry* and Yijan E. Chang

McDonnell Boehnen Hulbert & Berghoff LLP, Suite 3100, 300 S. Wacker Drive, Chicago, IL 60606, USA

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Abstract: Researchers in the field of tumor suppressor genes are actively attempting to discover new tumor suppressor genes and/or characterize known tumor suppressor genes with the intention of treating and diagnosing cancers. A number of recent patents and patent applications have been published that discuss some of these discoveries. Some of the patents and patent applications discuss newly discovered tumor suppressor genes, including WW Domain-Containing Oxidoreductase (WWOX), Cancer Associated Ring-1 (CAR-1), Human Cervical Cancer Suppressor 1 (HCCS-1), Src-suppressed C kinase substrate (SSECKS), ADP-Ribosylation factor-like putative Tumor Suppressor gene 1 (ARTS1), and Deleted in Osteosarcoma (DOS). One recent patent describes the discovery that known caspase family member caspase-8 (CASP8) is a tumor suppressor. Another recent patent describes the use of Wilms Tumor suppressor gene (WT1) peptides as a cancer vaccine. In addition, Sakai *et al.* received a patent describing a fragment of the p51 tumor suppressor gene containing a promoter region, which is useful for identifying compounds that modulate p51 activity. Another patent application recently published describes a chimeric tumor suppressor gene generated by combining a portion of the rat PEG-3 protein with the human GADD34 protein, thus creating a protein with apoptotic activity. These patents and patent applications provide valuable information that may be useful in fighting cancer by focusing on tumor suppressor gene activities.

Keywords: Tumor suppressor, tumor suppressors, homozygous deletion, methylation-induced gene silencing, gene therapy, WWOX, Caspase-8 (CASP8), Cancer Associated Ring-1 (CAR-1), Human Cervical Cancer Suppressor Protein 1 (HCCS-1), Wilms Tumor 1 (WT1), p51, SSECKS, Deleted in Osteosarcoma (DOS), ADP-Ribosylation factor-Like, putative Tumor Suppressor gene 1 (ARTS1).

INTRODUCTION

A tumor suppressor gene is a cellular gene whose biological role is to prevent normal cells from transforming into cancer cells. Loss of the tumor suppressor gene's function leads to tumor development. Tumor suppressor genes can be inactivated in a number of ways, including deletions of chromosomal locations where the genes reside and point mutations within the genes. Many tumor suppressor genes have been identified over the past several years. Some of the most well known and widely studied tumor suppressor genes include Rb (retinoblastoma), p53, DCC (deleted in colorectal cancer), APC (*Adenomatous polyposis coli*), WT1 (Wilms Tumor), NF1 (neurofibromatosis type 1), NF2 (neuro-fibromatosis type 2), VHL (von Hippel-Lindau), and MTS1 (multiple tumor suppressor 1, also known as p16INK4A) [1, 2].

Identification and characterization of tumor suppressor genes associated with particular cancers is advantageous for a number of reasons. Knowledge of the chromosomal location of a tumor suppressor gene can allow clinicians to screen patients for losses at that location to determine which gene may be contributing to or responsible for that patient's cancer. In addition, restoration of a lost or mutated tumor suppressor gene's function in a transformed cell may inhibit tumor growth. Restoring activity of a tumor suppressor gene may be accomplished by gene therapy techniques, for

example. Also, drugs that mimic or activate tumor suppressor genes in certain cells may be useful as therapeutic approaches for treating cancer.

Since the discovery and characterization of the first tumor suppressor gene, Rb (retinoblastoma), the attempt to discover additional tumor suppressors associated with various cancers has been steady. Several tumor suppressor genes have since been discovered and characterized, and the number of such genes being discovered continues to grow. Identifying tumor suppressors and associating them with particular cancers is an important endeavor for many reasons. For instance, understanding which tumor suppressor gene may be involved in a particular type of tumor cell growth enables researchers to develop treatments that may stop growth of that particular tumor cell, rather than using the current methods of chemotherapy and radiation that destroy normal cells along with tumor cells. In addition, since tumor suppressor gene inactivation is often caused by chromosomal deletions or translocations, diagnostics that identify such chromosomal abnormalities may assist clinicians in determining a proper course of treatment for a patient based on the particular type of tumor suppressor gene that may be inactivated in that patient's tumor cells.

The present article reviews a number of recently issued patents and recently published patent applications that have identified new tumor suppressor genes and uses thereof. The article is intended to be viewed as a starting point for further study of the genes discussed herein, and not a detailed analysis or historical overview of any particular tumor suppressor gene. In addition, the article discusses novel

*Address correspondence to this author at the McDonnell Boehnen Hulbert & Berghoff LLP, Suite 3100, 300 S. Wacker Drive, Chicago, IL 60606, USA; Tel: 312-913-3335; Fax: 312-913-0002; E-mail: derry@mbhb.com

approaches for studying tumor suppressor genes with the goal of providing clinically useful therapies for treating cancer patients. The article concludes with commentary on why researchers should monitor the patent landscape as it relates to tumor suppressor genes.

RECENTLY ISSUED PATENTS RELATING TO TUMOR SUPPRESSOR GENES

WWOX (WW Domain-Containing Oxidoreductase)

Aldaz and Bednarek describe the identification of a novel tumor suppressor gene referred to as WWOX [3]. The full length sequence of WWOX is shown in the reference [3] as SEQ ID NO: 1. WWOX has an open reading frame of 1245 base pairs, and encodes a WW-domain containing protein of 414 amino acids. The WWOX gene has nine exons and maps to human chromosome 16q23.3-24.1, which is known to be affected in a number of cancers, such as breast, liver, and prostate carcinomas.

The WWOX protein has two WW domains and a region that is highly homologous to the short-chain dehydrogenase/reductase family. According to Aldaz and Bednarek, WWOX is normally expressed in hormonally regulated tissues, including the testes, ovaries, and prostate. Based on this expression pattern and the relationship of WWOX to the short-chain dehydrogenase/reductase enzymes, Aldaz and Bednarek conclude that WWOX plays a role in steroid metabolism.

Aldaz and Bednarek demonstrate that WWOX can suppress tumor growth of breast tumor cells *in vitro* and *in vivo*. As discussed in Example 4 of the Aldaz and Bednarek patent, MDA-MB-435 and T47D cells, which express very low levels of WWOX, were transduced with WWOX cDNA [3]. Growth of the cells in soft agar was then observed to determine if expression of WWOX had any affect on cell growth. As expected, WWOX expression decreased the ability of these cells to grow in soft agar. To examine the effect of WWOX on tumor cells *in vivo*, MDA-MB-435 cells transduced with WWOX cDNA or vector alone as a control were injected into the mammary fat pads of nude mice. Growth of the tumor cells was monitored for seven weeks. Tumors formed in 10 of 10 injection sites of mice injected with the control cells, while 7 of 10 injection sites of mice injected with WWOX transduced cells formed tumors. In addition, Aldaz and Bednarek noted that the tumors generated from the WWOX transduced cells were much smaller than the tumors in the control mice.

On a mechanistic level, it has been shown that WWOX-1 protein is an essential mediator of tumor necrosis factor- α (TNF)-induced apoptosis [4]. It has been reported that hyaluronidase sensitizes tumor cells to TNF cytotoxicity via the action of WWOX-1. Hyaluronidase increases WWOX-1 gene and protein expression. Normally, a portion of cytosolic WWOX-1 is localized in mitochondria. TNF induces WWOX nuclear translocation by increasing mitochondria permeability and subsequent WWOX-1 nuclear localization. WWOX-1 enhances TNF or TRADD (TNF receptor associated death domain protein) mediated cell death partly by down-regulating Bcl-2 and Bcl-x and up-regulating p53. See Fig. (1). WWOX-1 is also shown to be an essential component in p53 mediated cell death [4].

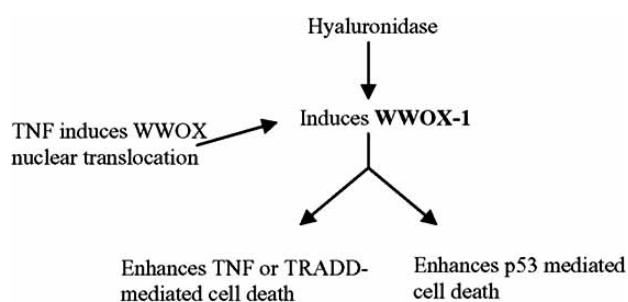


Fig. (1). Hyaluronidase sensitizes tumor cells to TNF cytotoxicity via WWOX-1.

The claims in the Aldaz and Bednarek patent cover “an isolated and purified polynucleotide comprising a nucleic acid sequence encoding” the WWOX protein, the amino acid sequence of which is shown as SEQ ID NO: 2 in the Aldaz and Bednarek patent [3]. The claims also encompass expression vectors that express the WWOX protein, recombinant host cells that comprise the WWOX protein, and a method of preparing the WWOX protein by transfecting a cell with a vector that expresses the protein.

Caspase-8 (CASP8)

Kidd *et al.* describe the discovery that caspase-8 (CASP8) is inactivated in cancers and has tumor suppressor activity [5]. As discussed by Kidd *et al.*, CASP8 is a cysteine protease that shares homology with the interleukin-1-converting (ICE)/caspase 1 gene family [5]. CASP8, caspase 9, and caspase 10 belong to a subfamily of caspases characterized by having duplicated death effector domains (DEDs) in a long pro-domain that precedes their cysteine protease catalytic domain. CASP8 can form a complex with an adapter molecule Fas-associated via Death Domain (FADD) through its DEDs. FADD has a death domain (DD) through which it associates with other cell death receptors, such as Fas, to form a death inducing signaling complex (DISC). Based on these characteristics, Kidd *et al.* indicate that CASP8 is upstream of other caspases and serves to activate caspase cascades that lead to cell death. See Fig. (2).

CASP8 can exert its apoptotic effect in a Fas-independent manner. It has been shown that the expression of CASP8 is suppressed during establishment of neuroblastoma metastases *in vivo* [6]. CASP8 seems to be an important determinant in cell survival during metastases, but not primary tumor growth in neuroblastoma cells. CASP8 expressing neuroblastoma cells undergo a CASP8 dependent apoptosis during tissue invasion in a death-receptor independent manner. The CASP8 mediated, Fas independent apoptosis is triggered by the presence of abnormal extracellular matrix, such as unligated integrins. This integrin mediated death occurs when CASP8 is recruited to and activated by clusters of unligated integrins at the cell surface. It was shown that the non-metastatic murine neuroblastoma cell line retained the expression of CASP8, whereas two metastatic sublines spontaneously derived from the original line had lost CASP8 expression [6].

Kidd *et al.* found that CASP8 is inactivated in a number of MYCN amplified neuroblastoma cell lines. The CASP8

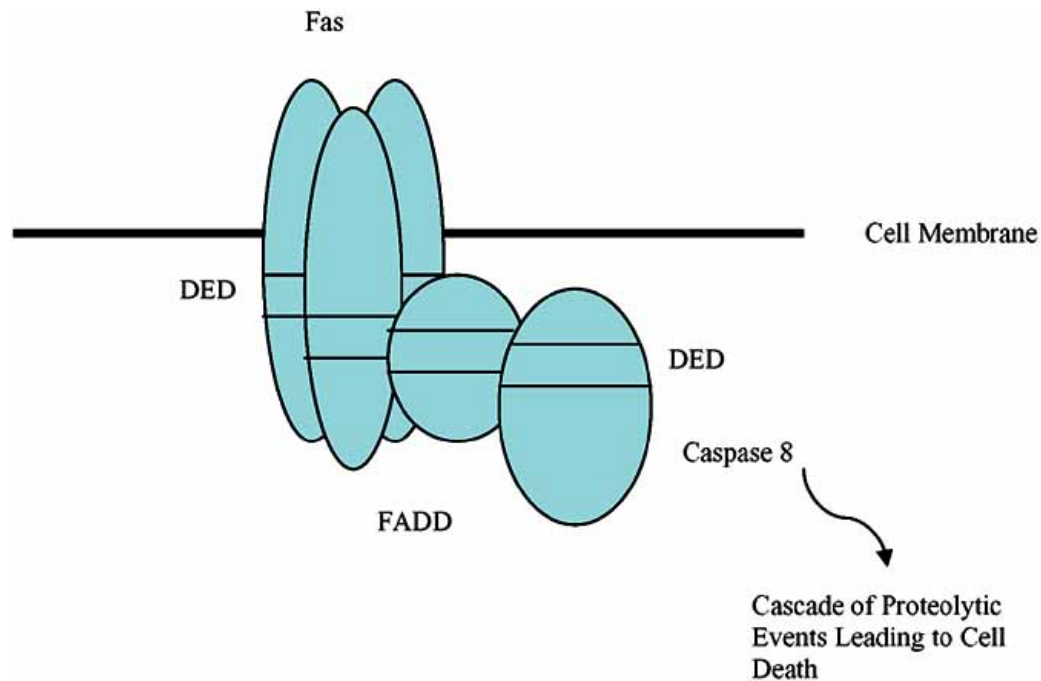


Fig. (2). Oligomerization of Fas via the death effector domain (DED) leads to the recruitment and activation of caspase 8 and cell death.

inactivation is described in the patent as occurring from homozygous deletion, heterozygous deletion with methylation-induced gene silencing, or homozygous methylation-induced gene silencing. Also, the patent indicates that CASP8 is inactivated in certain small-cell lung cancers, particularly those associated with amplified LMYC oncogene. The data in the patent supports the probability that CASP8 is inactivated in other cancers, especially those involving overexpression or amplification of MYC genes, such as NMYC, LMYC, and cMYC.

Kidd *et al.* disclose the two CASP8 promoter region sequences [5]. Kidd *et al.* indicate that these regions can be used for genomic methylation PCR analysis of CASP8 gene inactivation. The patent also describes specific primers for such methylation PCR analysis, shown in the patent as SEQ ID NO: 29 and SEQ ID NO: 34.

Based on Northern blot analysis of a selection of human adult tissues, CASP8 appears to be ubiquitously expressed. High levels of expression were seen in the brain, while very low levels of CASP8 were seen in the testis, skeletal muscle, and kidney. CASP8 expression was also noted in a number of other tissues, including human lymphoid tissues, such as spleen, lymph node, thymus, peripheral blood leukocytes (PBLs), bone marrow, and fetal liver, particularly those that undergo significant apoptosis.

Kidd *et al.* also show that CASP8 is localized to human chromosome 2q33-34. As discussed in the patent, this region of chromosome 2 is known to be involved in tumorigenesis of small-cell lung carcinoma, non-small-cell lung carcinoma, colorectal carcinoma, neuroblastoma, and uterine cervical carcinoma [5]. Thus, the localization of CASP8 is also consistent with its role as tumor suppressor. Based on the experiments in the patent, Kidd *et al.* indicate that the disclosed structure and localization of CASP8 can be used to

investigate various tumors for abnormal CASP8 structure and expression, thereby making CASP8 a useful diagnostic and/or prognostic marker for cancers, particularly for those associated with amplification of a MYC oncogene as discussed above.

The Kidd *et al.* patent claims methods for detecting inactivation of CASP8 in cancer cells, methods for prognosis of neuroblastoma, and methods for diagnosing aggressive neuroblastoma [5].

CAR-1 (Cancer Associated Ring-1)

Killary *et al.* describe the discovery of a tumor suppressor gene designated as CAR-1 (Cancer Associated Ring-1) [7]. The discovery of CAR-1 was accomplished by studying a genetic locus that was identified in Renal Cell Carcinoma (RCC). The locus, known as Nonpapillary Renal Carcinoma-1 (NRC-1), mediates suppression of tumor growth as well as cell death of various types of RCC [8]. A subtractive hybridization screening strategy identified one clone (CAR-1) that mapped to a different chromosome than the NRC-1 locus. While the NRC-1 locus is on chromosome 3p12, the CAR-1 gene maps to chromosome 1p31-1p36, which is known to show loss of heterozygosity in a number of cancers, such as neuroblastoma, breast carcinoma, and colon carcinoma [9-12].

The CAR-1 gene was then cloned and sequenced. The CAR-1 gene encodes two polypeptides based on splicing, including a 475 amino acid polypeptide, and a 304 amino acid polypeptide. The sequences of each CAR-1 protein are shown in the patent as SEQ ID NO: 1 (the 475 amino acid polypeptide) and SEQ ID NO: 2 (the 304 amino acid polypeptide) [7]. The amino acid sequence of CAR-1 indicated the presence of three RBCC domains. An RBCC domain includes an N-terminal RING finger Zn binding motif, a B-box Zn binding motif, and a helical coiled coil

domain [13]. Killary *et al.* also predicted that CAR-1 has a C-terminal Ret finger protein (rfp) domain.

The relationship of CAR-1 to the RBCC family further supported the likelihood that CAR-1 is a tumor suppressor gene, particularly since one of the RCC cell lines being investigated comprised a chromosomal translocation at a breakpoint at chromosome 1p. It is known that certain RBCC family members, as discussed by Killary *et al.*, can become fusion proteins as a result of chromosomal translocation, thereby making them oncogenic. For example, the promyelocytic leukemia (PML) gene fuses with retinoic acid receptor alpha (RARA) in acute promyelocytic leukemia; RET finger protein (Rfp) fuses to a tyrosine kinase domain to form the RET oncogene [14]; and Transcriptional Intermediary Factor 1 (TIF1) fuses to B-Raf proto-oncogene to become oncogenic [15]. Therefore, it is likely that chromosomal translocation causing CAR-1 to become a fusion protein can have an oncogenic effect.

A Northern blot analysis showed that CAR-1 is expressed in many tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes. Thus, it is likely that CAR-1 plays a universal role as tumor suppressor throughout many tissues in the body. To further demonstrate that CAR-1 is a tumor suppressor, Killary *et al.* examined the expression of CAR-1 in the five RCC cell lines, seven breast cancer cell lines, and three colon cancer cell lines. A decrease in CAR-1 expression or a loss of expression was found in one of the five RCC cell lines, three of the seven breast cancer cell lines, and in all three of the colon cancer cell lines. Expression was also analyzed in five colon tumor samples compared with adjacent normal colon tissue, and a decrease in CAR-1 expression was seen in all five cases.

The Killary *et al.* patent claims an isolated polynucleotide comprising the amino acid sequence of CAR-1, an expression cassette comprising the polynucleotide that encodes CAR-1, and a cell comprising the expression cassette [7]. The patent also discloses other uses of CAR-1, including methods for suppressing growth of cancer cells by contacting cells with the expression cassette that can express CAR-1 and diagnostic methods [7].

HCCS-1 (Human Cervical Cancer Suppressor 1)

Jin-Woo Kim describes the identification of a tumor suppressor protein designated Human Cervical Cancer Suppressor 1 (HCCS-1) [16]. The amino acid sequence of HCCS-1 is shown in the patent as SEQ ID NO: 2, and has a molecular weight of about 9 kilo Daltons [16]. The HCCS-1 gene is 555 base pairs in length, and has an open reading frame of 79 amino acids residues. The HCCS-1 DNA sequence is shown in the patent as SEQ ID NO: 1, and also has GenBank Accession No. AF249277 [16]. Kim indicates that the HCCS-1 gene is expressed in several normal human tissues, including cervical tissues, placenta, kidney, liver, skeletal muscle, and heart tissues. However, HCCS-1 is not expressed in cancer tissues such as primary cervical cancer and metastatic common iliac lymph node tissues, or in cancer cell lines such as promyelocytic leukemia HL-60 cells, HeLa cervical cancer cells, chronic myelogenous

leukemia K-562 cells, lymphoblastic leukemia MOLT-4 cells, Burkitt's lymphoma Raji cells, SW480 colon cancer cells, A549 lung cancer cells, and G361 melanoma cells.

Kim demonstrated that the HCCS-1 protein can inhibit growth of cervical cancer cells by transfecting HeLa cells with HCCS-1 gene. Kim demonstrated that HCCS-1 causes DNA fragmentation, cytoplasmic translocation of cytochrome c, membrane phosphatidylserine (PS) change, and apoptosis in HeLa cells. Specifically, Kim showed that the HCCS-1 protein of the invention can induce cytochrome c release from mitochondria to the cytosol causing DNA fragmentation, generates plasma membrane lipid changes (including translocation of phosphatidylserine (PS) of the inner leaflet into the extracellular side) leading to cell death, and can induce apoptosis in cells more sensitive to an apoptotic pathway as triggered by chemotherapeutic agents or radiation. In addition, it has been shown that cytochrome c released by HCCS-1 activates caspase-9 and -3 and finally results in cleavage of poly(ADP-ribose) polymerase [17]. The Kim patent mentions that HCCS-1 causes down-regulation of tumor-promoting proteins, for example, a mutant p53 tumor suppressor protein, Bcl-2 or c-Myc, although such experiments are not actually shown or described in Kim's patent.

The Kim patent claims "a polynucleotide which encodes a tumor suppressor protein and comprises the nucleotide sequence of SEQ ID NO: 1" [16]. The claims also encompass expression vectors that can express HCCS-1, isolated cells comprising HCCS-1 expressed by such expression vectors, and a particular cell that comprises the expression vector (*E. coli* JM109/HCCS1) [16].

WT1 (Wilms Tumor)

Sugiyama *et al.* discovered a tumor antigen that comprises a product of the Wilms tumor suppressor gene (WT1) [18]. The impetus behind the discovery was the desire to identify tumor antigens that can be used as a cancer vaccine against cancers that involve WT1 gene expression. As background, Sugiyama *et al.* discuss that cancer immunity occurs when killer T cells, which recognize a complex formed between the major histocompatibility complex (MHC) class I and a tumor antigen on a tumor cell [19-21]. Knowing that tumor antigens on the surface of tumor cells are generally about 8 to 12 amino acids in length, synthetic peptides were made of various lengths having 7 to 30 contiguous amino acids from WT1, all of the peptides having at least one amino acid known to be an anchor amino acid for binding to MHC.

The ability of the synthesized peptides to induce tumor immunity was tested *in vivo* using LPS-activated spleen cells pulsed with a WT1 peptide of the invention (see Example 8 in the patent) [18]. Specifically, mice were immunized once a week for three weeks, followed by the injection of leukemia cells. The mice immunized with the cells containing the WT1 peptide overcame and survived the tumor cell challenge, while the control animals (mice injected with LPS-activated spleen cells not expressing the WT1 peptide or injected with phosphate buffered saline only) died from the tumor challenge. The experiments described in the patent showed that the WT1 peptide of the

invention can function as a tumor antigen, and that it induced tumor cell specific killer T cells.

The claims of the Sugiyama *et al.* patent cover isolated polypeptides consisting of “at most 12 contiguous amino acid residues of a human WT1 protein, wherein the polypeptide comprises SEQ ID NO: 5, binds to MHC proteins, and the polypeptide induces killer T cells when bound to MHC class I antigen” [18]. SEQ ID NO: 5 is one of the particular synthetic WT1 peptides made and tested. The claims also encompass pharmaceutical compositions comprising the WT1 peptides of the invention, and methods of inducing killer T cells against cancer using the WT1 peptides of the invention [18].

p51

Sakai *et al.* discovered a fragment of the p51 tumor suppressor gene containing the p51 promoter region, as well as a gene encoding the p51 5'-untranslated region [22]. The p51 gene is a p53-like molecule as described by Osada *et al.* [23]. The sequence of the p51 promoter region is shown in US Patent No. 7,038,028 as SEQ ID NO: 1.

P51 is one of the isoforms expressed by the p63 gene, which shows strong homology to p53 tumor suppressor gene [24]. The isoforms that lack the N-terminal transactivation domain of p53 play an oncogenic, rather than a suppressive role in certain cancers [25]. P51 activates several, but not all p53-inducible genes, indicating that the mechanisms controlling p51- and p53-mediated tumor suppression differ [26].

The claims of the Sakai *et al.* patent encompass the isolated nucleic acid encoding the p51 promoter region and an isolated transformant or transductant having a plasmid that comprises the p51 promoter [22]. The data presented in the patent demonstrates that the isolation of the p51 promoter region is useful for diagnosis of certain cancers, and for screening of drugs that can modify the p51 promoter activity. Specifically, the patent describes a method of screening drugs using a cell line that retains a pGL2-neo/p51 promoter plasmid [22].

Table 1 provides a list of the issued patents discussed above and the tumor suppressor genes associated with each of the patents.

Table 1. Recent Patents Relating to Tumor Suppressor Genes

Gene Name	Inventors	Patent Number
WWOX	Aldaz and Bednarek	US7060811
Caspase-8	Kidd, Lahti, and Teitz	US7052834
CAR-1	Killary, Lott, and Chandler	US6943245
HCCS-1	Kim	US6977296
WT1	Sugiyama and Oka	US7030212
p51	Sakai, Kagaya, Sato, Sukenaga, Fujii, and Hideji	US7038028

RECENTLY PUBLISHED PATENT APPLICATIONS RELATING TO TUMOR SUPPRESSOR GENES

In addition to the above-discussed patented inventions involving tumor suppressor genes, the following patent applications have been published recently that relate to the discovery of novel tumor suppressor genes.

SSeCKS (Src-Suppressed C Kinase Substrate)

Gelman discloses a novel tumor suppressor gene called SSeCKS [27]. Gelman found that a SSeCKS gene encodes a substrate for protein kinase C that acts as a mitogenic regulator and a tumor suppressor. SSeCKS regulates cyclin D expression and localization, thereby mediating progression of cells from G1 through S phase of the cell cycle [27].

SSeCKS is a scaffolding protein for both protein kinase A and protein kinase C [28]. The SSeCKS gene is a rat ortholog of the human gene gravin, which is a member of the AKAP (A-kinase anchor proteins) family. AKAP member proteins direct the activity of protein kinase A by tethering the enzyme near its physiologic substrate [29].

The expression of SSeCKS is down-regulated in src- and ras-transformed fibroblasts. Re-expression of SSeCKS inhibits src-induced oncogenesis such as growth factor- and anchorage-independent growth, loss of contact inhibition, and metastatic potential. There is evidence showing that SSeCKS associates with cortical, actin-based cytoskeletal structures, and increases cell-cell adhesion in an inducible SSeCKS cell line. It is believed that SSeCKS reverses src-induced oncogenesis mainly by reorganizing actin-based cytoskeletal architecture and, subsequently, reestablishing feedback control of signaling pathways [30]. SSeCKS is also shown to regulate blood-brain barrier differentiation by modulating both brain angiogenesis and tight junction formation [31].

ARTS1 (ADP-Ribosylation Factor-Like, Putative Tumor Suppressor Gene 1)

Two patent applications published recently relate to the identification and cloning of the novel tumor suppressor gene designated ARTS1 (for ADP-Ribosylation factor-Like, putative Tumor Suppressor gene 1) [32,33]. ARTS1 (also designated ARLTS-1 or ARLS-1) is a novel member of the ADP-ribosylation factor family, and is located at chromosome 13q14. The ARTS1 cDNA has the GenBank accession number AF441378. ARTS1 expression was analyzed in 75 primary tumors and cell lines, which indicated that ARTS1 was downregulated by DNA hypermethylation in 25 (33%) of the human primary tumors and cell lines. In addition, 800 tumor and normal DNA samples were analyzed and several ARTS1 variants were identified. One of the variants is a germline nonsense polymorphism G446A (Trp 149Stop), which is three times more frequent in cancer patients who have a history of cancer in their family compared with the rest of the population. Also, the data in the patent application demonstrated that wild-type ARTS1 expression can suppress tumor formation of A549 cells, which normally have low expression levels of ARTS1. As discussed in the 20050266443 application, ARTS1 is the first ARF family member reportedly altered in human cancers. The patent application mentions that ARTS1 could

be involved in novel cytoplasmic/nuclear membrane trafficking and/or signaling cascades.

DOS (Deleted in Osteosarcoma)

Yajnik *et al.* disclose a novel tumor suppressor gene designated DOS (for Deleted in Osteosarcoma) [34]. Yajnik *et al.* also refer to the gene as DOCK 3 (Dedicator of Cytokinesis 3). In the application, representational Difference Analysis (RDA) on a mouse tumor model revealed a region of homozygous deletion in the mouse cell line that is homologous to human chromosome 7q31. The gene in the deleted segment was cloned from both mouse and humans. The human and mouse DOS protein sequence share about 97% identity. The application also indicates that DOS has about 30% identity with three known genes, including DOCK180, myoblast city, and Ced 5, all of which are involved in regulating actin cytoskeleton during cell migration. Based on the research described in the application, Yajnik *et al.* conclude that the DOS gene regulates actin cytoskeleton in cell growth and tumorigenesis.

PEG-3/GADD34 CHIMERA

One recently published application relates to chimeric tumor suppressor molecules comprising portions of rat PEG-3 ("rPEG-3") and human GADD34 ("hGADD34") that can have apoptotic activity [35]. As discussed in the patent application, a chimeric protein comprising amino acids 1-347 of rat PEG-3 fused with residues 418-674 of human GADD34 has anti-proliferative activity in transformed cells. The invention provides a number of chimeras, and encompasses the use of the chimeras for inhibiting cell proliferation, angiogenesis, and tumor growth.

Table 2 provides a list of the recently published patent applications discussed above and the tumor suppressor genes associated with each of the patent applications.

Table 2. Recent Patent Applications Relating to Tumor Suppressor Genes

Gene Name	Inventors	Application Number
SSeCKS	Gelman	US20050260693
ARTS1	Croce and Calin	US20050266443; US20060105340
DOS	Yajnik, Paulding, McClatchey, and Haber	US20060041111
rPEG-3/hGADD34 chimera	Fisher	US20060166220

CURRENT & FUTURE DEVELOPMENTS

The patents and applications reviewed above provide valuable lessons for those doing research in the field of tumor suppressors. For example, after years of discovering tumor suppressor genes, it is clear that others remain undiscovered. Thus, there remains an imperative need to continue searching for and characterizing new tumor suppressors. Also, studying known tumor suppressors can

lead to novel approaches for treating cancers. For example, Sugiyama *et al.* studied the well known WT1 gene and discovered that portions of the WT1 protein can be used to illicit an immune response against tumor cells. Thus, by studying portions of the WT1 protein, Sugiyama *et al.* have developed a vaccine against cancers associated with WT1 gene expression.

Also, understanding tumor suppressors can lead to cancer therapies for previously untreatable or hard to treat cancers. Tumor suppressor genes generally are involved in tumor growth because of inactivation, which can be caused, for example, by chromosomal deletion or mutations within the gene itself. Understanding what causes a tumor suppressor gene's inactivity is very important for developing therapeutic approaches to stopping tumor cell growth. For instance, in a tumor cell where the gene is deleted or otherwise silenced, gene therapy techniques could be used to express wild type tumor suppressors in the tumor cell. Where a tumor suppressor gene is not active because of a mutation, methods that restore normal activity or block aberrant activity of the mutant tumor suppressor gene may be warranted. Where gene therapy techniques are envisioned, the researcher should make every effort to test and demonstrate efficacy of the technique in an approved animal model.

Finally, identifying the chromosomal location of a tumor suppressor gene may aid in diagnosing cancers and help clinicians determine a proper course of treatment specific to a particular patient's cancer. For example, by detecting a chromosomal deletion associated with a particular tumor suppressor gene, a clinician would know that the tumor cells lack that particular gene in its active form. Thus, a therapy that restores the particular missing activity would more effectively target the patient's tumor cells, as opposed to treating the patient with a more general therapy, such as radiation or chemotherapy.

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