Is md-7/IL-24 a “Magic Bullet” for Cancer?

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Abstract

The “holy grail” of cancer therapy is to identify and exploit genetic elements and signal transduction pathways capable of selectively destroying tumor cells without eliciting harmful effects in normal cells or tissues. To achieve this objective, subtraction hybridization was combined with a “differentiation therapy” model of cancer in which human melanoma cells were induced to revert to a more “normal” state, growth arrest irreversibly, and terminally differentiate by treatment with fibroblast IFN and mezerein. This strategy permitted the cloning of a variety of genes involved in regulating important physiologic processes, including cell cycle, response to cytokines and viruses, tumorigenesis and metastasis, cancer growth control, apoptosis, and senescence. A specific gene, melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24), displaying cancer-specific apoptosis-inducing properties isolated using this scheme has now come into the limelight as a new gene therapy for divergent cancers. Although the mechanism of cancer cell selectivity of mda-7/IL-24 remains to be delineated, numerous attributes enable this gene as an effective therapy for cancer, including an ability to discriminate between normal and cancer cells, induce apoptosis in diverse tumor cells, promote “bystander” antitumor effects, inhibit tumor growth and angiogenesis in animal models, synergize with radiation, and modulate immune responses. These unique features combined with successful transition into the clinic instill confidence that mda-7/IL-24, as a single or more likely as part of a combinatorial approach, may provide profound therapeutic benefit for cancer patients. (Cancer Res 2005; 65(22): 10128-38)

Despite intense scrutiny, cancer remains a formidable disease with limited therapeutic options, especially in the context of metastatic disease (1). These findings underscore the imperative for improved methods to more effectively manage this potentially fatal ailment. In principle, gene therapy, involving selective replacement of tumor suppressor genes that promote tumor growth suppression or direct destruction, by programmed cell death or necrosis, or by employing a gene promoter that permits targeted virus replication and cytolysis in neoplastic cells represents treatment approaches with significant promise (2, 3). However, a number of factors restrict the effectiveness of this mode of therapy, including poor viral delivery to tumors, a paucity of tumor suppressor genes with cancer-selective activity, and immune clearance of therapeutic viruses (2, 3). This raises the following question: what would constitute the ideal tumor suppressor gene to employ for cancer therapy? Theoretically, a gene that promotes growth suppression and apoptosis only in cancer cells induces a potent “bystander antitumor effect” (thereby negating the problem of poor tumor transduction), synergizes with other treatment modalities (including radiation, chemotheraphy, and/or antibody-based therapies), augments immune processes (potentially further enhancing tumor cell destruction), and displays antiangiogenic properties (thereby inhibiting a tumor’s blood supply) would constitute an “ideal gene therapy for cancer.” Recent provocative studies by several laboratories using in vitro assays, animal tumor models, and recently a phase I clinical trial in patients with advanced cancers suggest that such a gene may actually exist, and this gene therapeutic is melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24; reviewed in detail in refs. 4–8). The purpose of this review is to provide a brief chronicle of the discovery, current understanding of the mode of action, and results of clinical studies of mda-7/IL-24, suggesting that this novel cytokine may indeed be the long sought and proverbial “magical bullet” for cancer.

Search for Genes Controlling Cancer Growth and Survival: Differentiation Induction Subtraction Hybridization

A question intriguing scientists is the nature of genes that discriminate between normal and cancer cells. Advances in molecular biology, including genomic microarrays and more recently proteomic arrays, are providing relevant insights into the plethora of genes altered as a consequence of cancer development and progression. Our studies in the 1990s sought to define genes whose expressions are altered as a consequence of reversion of cancer (melanoma) cells to a more “normal state” by induction of irreversible growth arrest and terminal differentiation (1, 9–13). A human melanoma cell culture system was identified that displayed induction of terminal differentiation following treatment with fibroblast IFN-β and the protein kinase C activating antileukemic agent, mezerein (14, 15). In this model system, a single treatment with IFN-β + mezerein for 24 hours results in irreversible commitment to terminal differentiation in essentially 100% of treated cells. With this model in hand, we developed and applied a then novel subtraction hybridization approach employing temporally spaced cDNA libraries, because we reasoned that multiple genes would change in a defined temporal manner during the differentiation process, to uncover genes involved in terminal differentiation and loss of proliferative capacity in human melanoma cells (9–11). This strategy was named differentiation induction subtraction hybridization (DISH), and the genes isolated were referred to as mda and DISH genes (Fig. 1; refs. 9–13). The power of this approach is emphasized by the spectrum of physiologically relevant genes that have been identified. These...
Figure 1. Schematic of DISH, an approach for identifying and cloning genes associated with induction of terminal differentiation in human melanoma cells. Treatment of HO-1 human melanoma cells with a combination of IFN-γ + mezerein results in a rapid and irreversible loss of proliferation, extinction of tumorigenic potential, and terminal differentiation (1, 2, 11–13). The DISH approach was developed to identify and clone genes associated with and causative of the physiologic changes associated with terminal differentiation. mRNAs were isolated from actively proliferating and IFN-γ + mezerein (2,000 units/mL + 10 ng/mL)-treated HO-1 cells that span the first 24 hours of treatment and were converted into cDNAs. Subtraction hybridization was then done between differentiation inducer-treated and control-proliferating cancer cells resulting in the production of a subtracted cDNA library enriched for mda genes. Probing of clones isolated from this cDNA library permitted the cloning of mda genes involved in critical cellular processes, some of which are listed here (1, 2, 11–13). Modified from Fisher et al. (6).
include the first cloning of p21 (referred to as mda-6; refs. 10, 16),
mda-5 (a novel IFN- and virus-inducible gene involved in apoptosis
and the innate immune response; ref. 17), mda-9 (syntenin, a gene
regulating many functions, including metastasis; ref. 18), human
polyadenylate phosphorylase (hPNPase\textsubscript{old-35}), a senescence-associated
and differentiation-associated gene; refs. 19–21), and mda-7/IL-24 (a novel cancer-specific apoptosis-inducing cytokine gene;
refs. 22–24).

**mda-7: A Novel Gene Associated with Melanoma Growth, Differentiation, and Progression**

An innovative and potentially less-toxic approach to cancer
treatment, which seeks to revert cancer cells to a more normal state
(i.e., “differentiation therapy”), is receiving increasing notice
(1, 8). The underlying premise of this strategy is that cancer cells
embody defects in normal programs of differentiation and treatment
with the appropriate agent(s) results in the induction of
threshold levels of gene products that restore normal growth control and induce terminal differentiation. In this context, genes of
potential relevance include those that when reactivated (or when
expression is enhanced) function as suppressors of the cancer phenotype, as well as genes that promote cancer apoptosis
(1, 9, 11). In human melanoma cells induced to terminally
differentiate, a direct corollary of the differentiation therapy
hypothesis is that specific genes expressed in actively proliferating
normal melanocytes would not be expressed or would be expressed
at reduced levels in melanoma cells (1, 9, 11). This was indeed the
case and initial applications of the DISH approach (Fig. 1) resulted
in the identification of mda-7 as a gene expressed in normal
melanocytes with decreasing mRNA expression during the process
of melanoma progression, from early radial growth phase, through
vertical growth phase to metastatic melanoma (8, 22). This
relationship was subsequently verified on a protein level in tissue
samples from patients with melanoma (25, 26). When melanoma
cells were treated with IFN-\(\beta\) + mezerein, mda-7 expression was
induced in a spectrum of melanoma cells, and this induction
related to irreversible growth suppression, induction of
melanin synthesis (a marker of melanoma differentiation), changes
in surface and intracellular antigen expression, and profound
alterations in gene expression (10, 14, 15, 22). These studies
provided proof-of-concept for differentiation therapy in human
melanoma and identified a target gene, mda-7, which was associated with the process of cancer reversion.

**Killing Diverse Cancers with Multiple Genetic Defects: Cancer-Specific Apoptosis-Inducing Properties of mda-7/IL-24 in a Broad Spectrum of Human Tumors**

Based on induction during terminal differentiation of human
melanoma cells, which correlated with an irreversible loss of
growth potential, we hypothesized that mda-7/IL-24 might display
an antiproliferative activity (9, 11, 22). This possibility was tested
and confirmed, initially in melanoma cells, showing growth-suppressing properties when ectopically expressed by means of
an expression vector (22). This observation provoked the obvious
question as to whether growth suppression was restricted to
melanoma cells or if this trait was more ubiquitous. Transfection of
mda-7/IL-24 into a broad range of human or rodent tumor cells
containing diverse genetic defects, including mutations in the
Rb, p53, and INK4A genes, decreased cell survival as monitored by
colony formation in monolayer culture (23). An intriguing finding
was that expression in normal fibroblast or epithelial cells did not
induce harmful effects (23).

To explore the apparent differential activity of mda-7/IL-24 in
cancer versus normal cells and to increase transduction of this
gene into target cells, we constructed a replication-incompetent
adenovirus expressing mda-7, Ad.mda-7/IL-24 (24). As observed with
transfection of mda-7/IL-24, Ad.mda-7 also inhibited growth and
decreased survival in a broad array of human tumor cells, without
eliciting detrimental effects in normal cells (refs. 24, 31–53;
Table 1). Studies in the context of human breast carcinoma cells
first documented that Ad.mda-7 induced apoptosis uniquely in
cancer cells irrespective of their p53 status (i.e., in p53 wild type,
p53 mutant, or p53-null breast carcinoma cells) and inhibited
breast tumor cell growth in vivo in athymic nude mice (24).
Moreover, these studies uncovered an important phenomenon
involving the bcl-2 family of proteins. Ad.mda-7 induced up-
regulation of the proapoptotic protein BAX, thereby shifting the
ratio of this proapoptotic protein to the antiapoptotic protein
BCL-2, supporting the hypothesis that an alteration in survival
signals induced by mda-7/IL-24 tilted the balance from survival to
death in breast cancer cells (24). Support for Ad.mda-7’s ability to
induce apoptosis and to alter bcl-2 family proteins, including
BCL-2, BCL-X\(_{L}\), BAX, and/or BAK, has now been verified in
multiple human cancers, including melanoma, glioblastoma
multiforme, mesothelioma, and carcinomas of the cervix, colon,
lung, nasopharynx, ovary, and prostate (24, 33, 34, 36, 39, 46, 53).
In addition to breast cancer (24), Ad.mda-7 also limits tumor
growth in animal models containing human breast, cervical,
colon, lung, and pancreatic carcinoma xenografts (31, 34, 35,
52, 53). These findings emphasize apparent ubiquitous antitumor
properties of Ad.mda-7 and provide further impetus to study this
intriguing gene and define its mode of action with the ultimate
aim of potentially translating this gene into the clinic to treat
patients with diverse cancers.
An Exception to the Ubiquitous Antitumor Properties of mda-7/IL-24: Pancreatic Cancer

Upon analyzing the antitumor properties of mda-7/IL-24 in diverse cancers, it became evident that infection of pancreatic cancer cells with doses of Ad.mda-7 that induced apoptosis in other tumor cell types failed to elicit growth inhibitory or apoptotic effects (34). This prompted investigations into the reason why this specific tumor subtype was refractory to Ad.mda-7 and to determine if this resistance could be reversed. We hypothesized that this intrinsic resistance might be a consequence of a specific genetic defect in this highly aggressive cancer. A conspicuous alteration in pancreatic cancers involves the Kirsten-ras (K-ras) gene, which is mutated in ~85% to 95% of pancreatic tumors and occurs early in the etiology of this disease (54–56). To ascertain the potential relevance of K-ras in mediating resistance to mda-7/IL-24, we targeted the K-ras gene for extinction using phosphorothioate oligonucleotides or by means of an expression vector expressing K-ras antisense (34). Using this strategy, Ad.mda-7 was capable of inducing apoptosis uniquely in mutant K-ras-expressing pancreatic tumor cells (34). This combinatorial effect highlighted an interesting phenomenon, infection with Ad.mda-7 resulted in robust levels of mda-7/IL-24 mRNA, with minimal translation into MDA-7/IL-24 protein. However, after nullifying K-ras expression, an abundant MDA-7/IL-24 protein was produced. This finding emphasizes an interesting process, recently shown with other strategies to transduce the entire tumor mass with the suppressor gene, that induced apoptosis in mda-7/IL-24 and inhibition of mutant K-ras gene. Moreover, initial studies in pancreatic cancer cells highlighted significant bystander antitumor activity of mda-7/IL-24 (34). In this model system, transfection with a K-ras antisense expression vector or treatment with antisense phosphorothioate oligonucleotides followed by infection with Ad.mda-7 resulted in a synergistic decrease in mutant K-ras pancreatic tumor colony formation (34). Moreover, this combinatorial approach (i.e., transfection with K-ras antisense plus infection with Ad.mda-7) also resulted in a loss of tumorigenic potential in mutant K-ras AsPC-1 pancreatic tumor cells, despite the fact that at most only 8% to 10% of cells received both agents. Further confirmation and documentation of bystander antitumor activity has now been obtained in several model cell culture systems, both tumor and normal, and in phase I clinical trials involving direct intratumoral injection with mda-7/IL-24.

Making a Good Therapeutic Even Better: Profound “Bystander Antitumor” Activity of mda-7/IL-24

The ability to effectively employ tumor suppressor gene replacement therapy for cancer has limitations. One of these involves the inability using current viral and nonviral methodologies to transduce the entire tumor mass with the suppressor gene, a problem that is further intensified in the context of metastatic lesions (2, 3, 49, 52, 53, 58). Based on this consideration, a tumor suppressor gene, which functions as a cytokine and can induce anticancer properties not only in target cells receiving the suppressor gene but also in adjacent and distant tumor cells (i.e., a “bystander antitumor” activity), provides a means of obviating this technical difficulty (34, 50, 59). Several studies employing diverse cancer and normal cell systems confirm that mda-7/IL-24 is secreted from both types of cells following infection with Ad.mda-7 (33, 36, 40, 50, 59). Moreover, initial studies in pancreatic cancer cells highlighted significant bystander antitumor activity of mda-7/IL-24 (34). In this model system, transfection with a K-ras antisense expression vector or treatment with antisense phosphorothioate oligonucleotides followed by infection with Ad.mda-7 resulted in a synergistic decrease in mutant K-ras pancreatic tumor colony formation (34). Moreover, this combinatorial approach (i.e., transfection with K-ras antisense plus infection with Ad.mda-7) also resulted in a loss of tumorigenic potential in mutant K-ras AsPC-1 pancreatic tumor cells, despite the fact that at most only 8% to 10% of cells received both agents. Further confirmation and documentation of bystander antitumor activity has now been obtained in several model cell culture systems, both tumor and normal, and in phase I clinical trials involving direct intratumoral injection with mda-7/IL-24 as a Magic Bullet for Cancer

Table 1. Ad.mda-7 selectively inhibits growth and induces apoptosis in a wide spectrum of human cancer cells without affecting normal cells

<table>
<thead>
<tr>
<th>Normal cells (growth and viability not affected)</th>
<th>Tumor cells (growth inhibited, apoptosis induced)</th>
</tr>
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<tbody>
<tr>
<td>Mammary epithelial cells</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Prostate epithelial cells</td>
<td>Prostate carcinoma</td>
</tr>
<tr>
<td>Melanocytes</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Bronchial epithelial cells</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>Fetal astrocytes</td>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td>Skin fibroblasts</td>
<td>Osteosarcoma</td>
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<tr>
<td>Lung fibroblasts</td>
<td>Colon carcinoma</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Renal epithelial cells</td>
<td>Pancreatic carcinoma*</td>
</tr>
<tr>
<td>Ovarian epithelial cells</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>Mesothelial cells</td>
<td>Ovarian carcinoma</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>Endothelial carcinoma</td>
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<tr>
<td>Neuronal cells</td>
<td>Renal carcinoma</td>
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<td></td>
<td>Hepatocarcinoma</td>
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<td></td>
<td>Neuroblastoma</td>
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*In pancreatic carcinoma cells containing a mutant K-ras gene, the combination of Ad.mda-7 and inhibition of mutant K-ras induces growth inhibition. Ad.mda-7 alone or in combination with inhibition of mutant K-ras fails to induce growth inhibition or apoptosis in BxPC-3 pancreatic carcinoma cells, which contain a wild-type K-ras gene.
Ad.mda-7 (6, 7, 50, 52, 59–61). Confirmation of a bystander antitumor effect of mda-7/IL-24 in vivo has now been documented in animal models using T47D breast carcinomas established on opposite flanks of nude mice, to approximate a metastatic state (52). Administration of Ad.mda-7 intratumorally to tumors on the left flank affected tumor growth on the right flank, thereby providing direct evidence that Ad.mda-7 when administered to a tumor can enter the circulation and exert a biological effect on neoplastic cells located at a distant site. The mechanism underlying this bystander antitumor activity of mda-7/IL-24 is not known but may involve direct antitumor cytotoxic or antiangiogenic activity of secreted MDA-7/IL-24 protein and/or stimulation of immune-mediated antitumor activity (6–8, 27, 28, 50, 52, 59).

An interesting recent study supports the potential use of normal cells as a reservoir for producing and secreting mda-7/IL-24, which can then induce cancer cell–specific death, an inhibition in tumor cell invasion, and enhanced sensitivity of cancer cells to radiation-induced apoptosis (50). These recent studies highlight the importance of IL-20/IL-22 surface receptors (discussed below) in mediating this “bystander” activity in cancer cells. This significant property of mda-7/IL-24 to promote activity as a secreted molecule, including an ability to augment sensitivity to radiation (discussed below), emphasizes its truly unique potential as a gene therapy for cancer.

**Complexity Rules, Part 1: Multiple Signaling Pathways Regulate mda-7/IL-24-Induced Apoptosis in Cancer Cells**

The role of defined signal transduction pathways in mediating the cancer-selective apoptosis properties of mda-7/IL-24 has been investigated (reviewed in refs. 4, 8). These studies underscore the complexity of action of this novel cytokine. Initial studies in human melanoma, subsequently confirmed in malignant glioma and prostate carcinomas, confirm a role of p38 mitogen-activated protein kinase and induction of the growth arrest and DNA damage inducible gene (GADD) family, including GADD34, GADD45, and GADD153, in selective induction of apoptosis (37). Studies in human non–small cell lung carcinomas (NSCLC) call attention to a role for PKR in Ad.mda-7-induced apoptosis in this tumor model (62). Additionally, an involvement of c-Jun NH₂-terminal kinase (JNK) kinase activation, specifically in the context of enhancing radiation sensitivity by mda-7/IL-24, has been documented in several model systems, including NSCLC, malignant gliomas, and prostate carcinomas (44, 63, 64). Recent studies in pancreatic cancer cells show an ability of ROS generators, including arsenic trioxide, N-(4-hydroxyphenyl), and dithiophene (NSC656240), to synergize with Ad.mda-7 in inducing apoptosis and confirm the complexity of signal transduction pathway changes culminating in apoptosis in different pancreatic cancers (48). Similarly, in the context of ovarian cancer, activation of the intrinsic mitochondrial death pathway seems to be the predominant pathway involved in Ad.mda-7-induced apoptosis (47), whereas in a specific ovarian carcinoma cell line (MDA-H 2274), the extrinsic death receptor pathway has been implicated in Ad.mda-7-induced apoptosis (51). The totality of the present data supports the hypothesis that mda-7/IL-24 can affect multiple signaling pathways in cancer cells ultimately eliciting biochemical changes that promote apoptosis, uniquely in the context of tumor cells (refs. 4–8; Fig. 2). Distinctively, mda-7/IL-24 identifies a kink in the armor of tumor cells and exploits this defined weakness resulting in programmed cell death.

**Complexity Rules, Part 2: mda-7/IL-24 Can Induce Apoptosis by an Intracellular Process in a Janus-Activated Kinase/Signal Transducers and Activators of Transcription–Independent Manner**

The canonical mechanism by which diverse cytokines, including members of the IL-10 gene family, are proposed to mediate bioactivity is by binding to defined cell surface receptors and activating Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathways (29, 30). In the case of mda-7/IL-24, JAK/STAT activation is induced after binding of MDA-7/IL-24 protein to the IL-20R1/IL-22R1 and IL-20R2/IL-22R1 receptors (65, 66). In the context of mda-7/IL-24’s “antitumor bystander” activity, these receptor interactions are clearly relevant and important for mediating activity of this cytokine (50, 59). Considering this possible mode of action relative to induction of programmed cell death by mda-7/IL-24, we investigated whether cancer cell–specific apoptosis requires a complete complement of receptors and/or JAK/STAT signaling (41). These studies resulted in the intriguing and unanticipated observation that cell killing by mda-7/IL-24 did not require functional cell surface receptors or STAT activation and inhibiting tyrosine kinase activity or infection of cancer cells defective in specific components of the JAK/STAT signaling pathway still elicited apoptosis (41). Additionally, using an adenovirus expressing mda-7/IL-24 without its secretory signal (Ad.SP-mda-7), thereby resulting in cellular accumulation in the absence of secretion, we directly confirmed that apoptosis induction occurred in cancer cells without the need for secretion (67). These findings have been confirmed and expanded using a GST-MDA-7 fusion protein (42) and transfection approaches (68), uncovering a unique mode of action of mda-7/IL-24 involving an intracellular as well as a secreted extracellular mode of anticancer action (Fig. 3).

The ability of nonsecreted MDA-7/IL-24 protein to induce apoptosis in cancer cells is fascinating and suggests potential intracellular targets as mediators of cancer-specific antitumor activity of this novel cytokine. Based on localization of MDA-7/IL-24 in the endoplasmic reticulum (ER), it is possible that interactions between MDA-7/IL-24 and specific target molecules in the ER of cancer cells elicit an unfolded stress response (42, 67), thereby promoting cancer cell apoptosis. The identity of the primary targets in the ER and what accessory molecules, if any, present in the cancer cell that are direct regulators of cell killing remain to be elucidated. An interesting question that also remains to be resolved is why a similar accumulation of intracellular MDA-7/IL-24 protein in normal cells does not elicit apoptosis. Further studies are necessary to clarify these issues and to determine if the target or accessory molecules in normal cells are altered or less abundant, whether a similar unfolded stress response is even elicited in normal cells, and/or whether an unfolded stress response is induced in normal cells; however, they are simply better adapted to handle this cellular change. These intriguing questions are currently being investigated and may lead
to the development of innovative approaches for enhancing the therapeutic activity of MDA-7/IL-24.

**Attacking Cancer on Many Fronts: mda-7/IL-24 Inhibits Tumor Angiogenesis**

Angiogenesis, production of new blood vessels, is a key component in tumor expansion and metastasis (69). This process is essential for tumor growth beyond a certain volume and for the development of metastases (69). In these contexts, selected inhibition of angiogenesis in tumors provides a viable strategy, which is currently being evaluated in the clinic, for inhibiting tumor growth and enhancing patient survival. In initial studies investigating the effect of mda-7/IL-24 in tumor formation in nude mice injected with Ad.mda-7, an inhibition of tumor growth was associated with a decrease in blood vessel formation (35). This observation suggested that in addition to its ability to directly kill tumor cells, mda-7/IL-24 might also delimit tumor growth by affecting its blood supply. Further studies confirmed and expanded on these initial studies, indicating that mda-7/IL-24 was a potent inhibitor of angiogenesis, and this effect was mediated by secreted MDA-7/IL-24 affecting endothelial cells through interactions with the IL-20R1/IL-20R2 receptors on specific cells in the immune system, resulting in the release of secondary cytokines, including tumor necrosis factor-α (TNF-α), IFN-γ, IL-6, IL-1β, IL-12, and granulocyte macrophage-colony stimulating factor (GM-CSF). These secondary cytokines, as well as MDA-7/IL-24, might then elicit additional changes in cellular physiology, including antitumor immune responses, induction of cell proliferation in specific immune cell subsets, and/or effects on immune cell growth (including alterations in differentiation status, either maintenance or inhibition). Supraphysiologic levels. Infection of tumor cells with Ad.mda-7 is a potent inducer of apoptosis, promoting this effect selectively in a broad range of human cancers without eliciting harmful effects in normal cells (Table 1). Induction of programmed cell death associates with a plethora of biochemical changes promulgated by mda-7/IL-24 in specific cancer cells, including phosphorylation of double-stranded RNA-dependent protein kinase R (PKR), p38 mitogen activated protein kinase (MAPK), extracellular regulated kinase 1/2 (ERK 1/2), and eukaryotic translation initiation factor-2α (eIF2α); down-regulation of inducible nitric oxide synthase (iNOS); up-regulation of proapoptotic proteins (Bax and Bak); down-regulation of antiapoptotic proteins (Bcl-2 and Bcl-xL); and induction of the Fas/FasL pathway. A key component in the apoptotic cascade involves induction of an ER stress response in cancer cells, which can provoke the induction of the GADD family of genes, as well as phosphorylation of p38 mitogen activated protein kinase and eukaryotic translation initiation factor-2α. These changes have been shown to associate with induction of apoptosis by mda-7/IL-24 in several tumor cell types. Administration of mda-7/IL-24 by means of Ad.mda-7 also inhibits tumor cell growth, invasion, and migration, which may involve effects on the β-catenin and/or the phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB) pathways. Additionally, Ad.mda-7 can affect angiogenesis (new blood cell formation) by inhibiting endothelial cell differentiation and by blocking VEGF or transforming growth factor-β (TGF-β) activity by inhibiting src activity in tumor cells. Modified from Biotechniques 2002 Oct Suppl: 30-9 with permission of Eaton Associates.
angiogenesis directly and indirectly by inhibiting tumor cell VEGF production. The importance of inhibition of angiogenesis by mda-7/IL-24 was further highlighted in studies combining radiation and Ad.mda-7 to treat A549 NSCLC cells in nude mice (72). A549 cells lack a complete set of functional IL-20/IL-22 receptors (50, 59), thereby preventing secreted MDA-7/IL-24 from directly inducing biological effects on this cell line, including inhibition of invasion, augmentation of radiation lethality, and induction of cell death (50). However, infection of A549 cells with Ad.mda-7 resulted in secreted MDA-7/IL-24, which potentiated the ability of radiation to inhibit endothelial cell survival, resulting in a profound antitumor activity of this combinatorial approach in animals (72). Taken together, these observations suggest that MDA-7/IL-24 will have an expanded scope of therapeutic activity in vivo by provoking direct tumor cell killing in cancer cells containing functional IL-20/IL-22 receptors and when employed in combination with radiation by inhibiting tumor growth in cancer cells containing or lacking functional IL-20/IL-22 receptors through inhibition of angiogenesis.

Enhancing MDA-7/IL-24 Anticancer Activity: Radiation, Chemotherapy, and Monoclonal Antibodies Increase MDA-7/IL-24 Antitumor Activity

Treatment options for cancer patients frequently involve surgery combined with various therapeutic approaches, including radiation, chemotherapy, immunotherapy, and recently antiangiogenic therapy (1, 69). In this context, methods enhancing the effectiveness of anticancer agents, without promoting toxicity, would be of immense use and could provide a means of significantly improving clinical responses. Studies in NSCLC (63) and malignant gliomas (40, 44–46) formally showed that radiation enhances tumor cell sensitivity to mda-7/IL-24. In both model systems, one employing normal lung fibroblast cell lines and the other using primary and immortal human fetal astrocyte cell cultures, growth inhibition and induction of apoptosis did not ensue following treatment with similar doses of radiation combined with mda-7/IL-24. These studies highlight the specificity of action of this combinatorial approach and also indicated the importance of JNK activation as a mediator of radiation enhancement of mda-7/IL-24 lethality in cancer cells.

Figure 3. Model of the possible molecular basis of mda-7/IL-24 cancer cell–mediated apoptosis. Effects of known physiologic (left) and ectopic (right) overexpression of mda-7/IL-24. Normally, MDA-7/IL-24 binds to cognate receptors and activates STAT1 and STAT3 transcription factors to mediate pathways affecting cell growth. Because mda-7/IL-24 mRNA and protein are normally seen in subpopulations of immune cells and melanocytes, effects are likely initiated in these cell types but might also affect neighboring nonproducing cells because the protein is secreted. When normally or ectopically overexpressed, current findings indicate that MDA-7/IL-24 localizes to the ER/Golgi compartments, whether or not the protein contains a secretory signal. Accumulation of MDA-7/IL-24 protein in this compartment triggers apoptosis that could apparently involve induction of pathways described currently as ER stress. However, MDA-7/IL-24 additionally acts indirectly on mitochondria to generate reactive oxygen species. Secreted MDA-7/IL-24 protein employs the IL-20R/IL-22R receptors to activate signal transduction pathways and/or potentially enter cancer cells and activate proapoptotic pathways by localization and accumulation in the ER/Golgi compartment and/or by inducing mitochondrial dysfunction. A combination of pathways triggered by mda-7/IL-24 results in transformed cell-specific apoptosis. Modified from Sauane et al. (67).
NSCLC and malignant glioma cells (40, 46, 63). A detailed discussion of potential mechanisms underlying this synergistic interaction between mda-7/IL-24 and radiation can be found in Dent et al. (73) and Gupta et al. (8).

In two recent studies, we uncovered an important property of mda-7/IL-24: an ability to induce a potent radiation enhancement “bystander antitumor effect” not only in cancer cells inherently sensitive to this cytokine but also in tumor cells overexpressing the antiapoptotic proteins BCL-2 or BCL-2, and exhibiting resistance to the cytotoxic effects of both radiation and MDA-7/IL-24 (50, 64). These recent studies done in prostate carcinoma cells also reinforced the importance of JNK activation in facilitating the ability of radiation to synergize with MDA-7/IL-24 in inducing enhanced lethality of these agents in cancer cells (64). These findings offer promise for greatly expanding the use of mda-7/IL-24 for cancer therapy, especially in the context of radiation therapy. Additionally, recent reports indicate that Ad.mda-7 lethality in NSCLC cells can also be augmented by combination treatment with the nonsteroidal anti-inflammatory drug sulindac (74), and that Ad.mda-7 amplifies the antitumor effects of Herceptin (trastuzumab), an anti-p185<sup>erbB2</sup> murine monoclonal antibody (mAb) that binds to the extracellular domain of ErbB2, in breast carcinoma cells overexpressing HER-2/neo (75). Overall, these studies employing radiation, a chemotherapeutic agent, and a mAb reinforce the possibility of augmenting the anticancer activity of mda-7/IL-24 and increasing its therapeutic index as a gene therapy for diverse cancers by using combinatorial therapy approaches.

A Novel Approach for Augmenting MDA-7/IL-24 Therapeutic Activity: Cancer-Selective Virus Replication Targeting mda-7/IL-24 Gene Delivery

Targeting gene expression in cancer cells provides a unique opportunity for controlling viral replication and for delivering genes to tumor cells (3, 49, 52, 53, 58, 76). To optimize this approach, identification of promoter elements that can differentiate between normal and cancer cells is of immense import (49, 52, 58, 76). One such promoter element, which is showing promise in this regard, is the telomerase promoter (76). Using subtraction hybridization, we identified a novel gene, progression elevated gene-3 (PEG-3), whose expression is enhanced in rodent tumors displaying an aggressive transformed phenotype, oncogenically transformed rat embryo fibroblast cells, and DNA-damaged rodent cells (77, 78). Recent studies now confirm that PEG-3 is a mutated and truncated form of rat GADD34 that develops frequently during the process of rodent tumor formation and which functions as a dominant-negative mutant of both rat and human GADD34 genes (79).

To define the mechanism of regulation of the PEG-3 gene, we isolated its promoter (PEG-Prom) and characterized its expression profile and the factors involved in its regulation (80, 81). These studies identified the transcription factors activator protein 1 (AP-1) and PEA-3 as primary regulators of transcriptional regulation in rodent tumor cells (80, 81). Because these factors are frequently up-regulated in human cancers, we evaluated the full-length and various mutated regions of the PEG-Prom for activity in human tumor cells (49, 52, 58). These studies indicated that the PEG-Prom and specific deletion mutants had robust activity in diverse human tumors, with limited activity in normal cells (49, 52, 58). Using a partial PEG-Prom element, replication incompetent adenoviruses were constructed in which mda-7/IL-24 or wild-type p53 expression was regulated in a cancer-specific manner by the PEG-Prom (49). When administered to human prostate tumor cells, apoptosis was induced, whereas no toxic effect was observed in normal cells.

To expand on these observations, we constructed bipartite adenoviruses in which the PEG-Prom regulates viral replication and the cytomegalovirus (CMV) promoter regulates mda-7/IL-24 (52) or IFN-γ expression (58). These viruses selectively replicated in cancer cells resulting in direct cytolysis and augmented antitumor activity in vivo in animal models as a consequence of targeted induction of mda-7/IL-24 or IFN-γ expression concomitant with virus replication. Using experimental models in which tumors were established on opposite flanks of an animal (to mimic the metastatic state) and the therapeutic virus was administered only to tumors on one side of the animal, complete cures resulted as indicated by an absence of detectable tumors on either flank using human breast tumor xenografts, Ad.PEG-E1A.mda-7 (52), or pancreatic carcinoma tumor xenografts, Ad.PEG-E1AIFN-γ (58). In contrast, viruses expressing solely replicative functions resulting from the CMV promoter or PEG-Prom driving Ad.E1A and Ad.E1B expression and nonreplicating viruses expressing mda-7/IL-24 or IFN-γ did not elicit a similar profound antitumor activity on noninjected tumors. Additionally, a recent study using a modified adenovirus that replicates preferentially in p53 mutant cells and expresses mda-7/IL-24 (ZD55-IL-24) also produced an enhanced antitumor effect in nude mice containing a human colon carcinoma xenograft compared with nonreplicating viruses expressing mda-7/IL-24 (53). These provocative studies indicate that administering mda-7/IL-24 using conditionally replicating cancer-specific adenoviruses represents a potentially viable strategy for increasing the therapeutic efficacy of this novel cytokine.

mda-7/IL-24 Successfully Enters the Clinic: Documentation of Safety and Early Signs of Efficacy as a Gene Therapy for Cancer

Based on its wide-ranging activity in multiple in vitro cancer cell culture systems, which translated into profound anticancer activity in nude mouse human tumor xenograft models, mda-7/IL-24 has now taken the noteworthy and mandatory step for a putative cancer gene therapy by entering the clinic (6, 7, 60, 61). These studies are extremely exciting, indicating that mda-7/IL-24 when administered intratumorally using a replication incompetent adenovirus is safe and displays genuine signs of clinical efficacy (6, 7, 60, 61). Moreover, the results obtained in patients in this phase I clinical trial recapitulated many of the observations initially uncovered using cell culture and animal tumor models (i.e., effective induction of tumor cell-specific apoptosis, potent antitumor bystander effect, and immune modulation; reviewed in detail in refs. 7, 8). In this trial, 28 patients with resectable solid tumors received intratumoral injections with Ad.mda-7 (INGN 241, 2 × 10<sup>16</sup> to 2 × 10<sup>12</sup> viral particles; ref. 60). In 100% of injected lesions, vector transduction, transgene mRNA, elevated MDA-7/IL-24 protein, and apoptosis induction were evident. In specific instances, a single injection with Ad.mda-7 (INGN 241) resulted in transduction of 10% to 30% of the tumor mass with ~70% of the tumor cells displaying signs of apoptosis, supporting the antitumor bystander effect observed originally in cell culture models (34, 50, 59). Multiple injections with Ad.mda-7 (INGN 241) were found to be safe with toxicity being self-limiting and generally mild. Moreover, the conclusion from this study was that mda-7/IL-24 was well tolerated, capable...
of inducing apoptosis in a significant portion of tumor cells, and this therapy displayed evidence of clinically significant activity (7, 60). Additionally, this phase I clinical trial (61) also supported the immune-modulating properties of mda-7/IL-24, including transient increases in serum levels of IL-6, IL-10, and tumor necrosis factor-α with marked increases in CD3+CD8+ T cells (suggesting enhanced T effector cytokine production and activated CD8+ T cells). Taken together, these findings engender optimism that mda-7/IL-24 may provide significant benefit for patients with multiple types of cancer, especially in the context of improved tumor delivery (52, 53) and by applying combinatorial approaches (34, 38, 40, 43–46, 48, 50, 63, 72, 74, 75).

Summary and Future Perspectives

In an unprecedented time frame, mda-7/IL-24 has progressed from cloning as a novel gene induced during terminal differentiation of melanoma cells to elucidation of cancer-specific apoptosis-inducing properties in vitro in cell culture and in vivo in animal models to documentation of safety and clinical activity in patients with advanced carcinomas and melanomas (reviewed in detail in refs. 5–8, 73, 82). This remarkable journey has revealed a number of interesting and unique properties of this IL-10-family member cytokine, including direct cancer-killing properties, lack of toxicity in normal cells, potent bystander antitumor activity, immune-modulating activity, and antiangiogenic properties. In the realm of antitumor agents, mda-7/IL-24 is truly unique, displaying wide spectrum, selective antitumor activity in cancer cells containing multiple genetic abnormalities (Table 1) and having the capacity to exploit diverse signaling pathways in mediating tumor cell death (Fig. 2). Although a cytokine that is capable of interacting with and signaling through the IL-20/IL-22 receptor complex, mda-7/IL-24 exploits a unique method of cell killing that occurs in both a JAK/STAT- and receptor-independent manner (Fig. 3). These observations suggest a novel method of action of mda-7/IL-24 that may involve specific intracellular targets that remain to be elucidated. The ability to augment the antitumor properties of this cytokine by using radiation, ROS inducers,
anticancer drugs, and mAbs highlights innovative ways of potentially improving clinical efficacy of mda-7/IL-24. Similarly, using conditionally replicating adenoviruses that specifically target cancer cells to deliver mda-7/IL-24 improves the therapeutic activity of this gene, already evident in breast and colon carcinomas and likely extendable to multiple additional neoplasms (Fig. 4). Another innovative approach requiring further refinement and evaluation involves the use of normal cells, including tumor microenvironment and stem cells, as reservoirs and delivery vehicles for MDA-7/IL-24 proteins (Fig. 4). The approaches briefly outlined above could prove amenable for the therapy of metastatic disease, especially by employing combinatorial treatment protocols, such as radiation, chemotherapy, or mAb therapy with mda-7/IL-24 delivery. The mda-7/IL-24 story is an exciting work in progress as highlighted in this and other reviews (5–8, 73, 82); the future seems optimistic for this unique cytokine and only time will tell if it is indeed a “magic bullet” for treating diverse cancers.

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27. Huang EY, Madireddi MT, Gopalakrishnan RV, et al. Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppression as highlighted in this and other reviews (5–8, 73, 82); the


Is *mda-7/IL-24* a "Magic Bullet" for Cancer?

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