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Differentiation Therapy of Cancer: Journey from the Laboratory into the Clinic

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ABSTRACT

Cancer is a progressive process characterized by uncontrolled cell proliferation and de-differentiation and clinical protocols involving cytotoxic agents remain a mainstay of conventional cancer therapies despite many non-specific adverse side effects. An alternative approach to potentially reduce the toxicity of anti-cancer therapy employs the induction of cancer cells to undergo terminal differentiation leading to irreversible inhibition of growth and induction of programmed cell death (apoptosis). The concept of ‘differentiation therapy of cancer’ has been validated using cell culture and animal models including leukemia, neuroblastoma and melanoma supporting its potential for translation into the clinic. By inducing terminal differentiation of metastatic human melanoma cells in combination with subtraction hybridization, we have identified and cloned novel genes that participate in critical cellular processes including genes involved in cell cycle and growth control, differentiation, metastasis, innate immune response, apoptosis, inflammation and senescence. One originally novel gene, melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24) is a member of the IL-10 gene family of cytokines and is a cancer cell-specific inducer of apoptosis. This review discusses the concept of ‘differentiation therapy of cancer’ in a historical context and highlights important findings from the melanoma model system with an emphasis on the translation of basic research findings to the clinical treatment of cancer patients.

Keywords: terminal differentiation, cancer, leukemia, melanoma, mda-7, IL-24
Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ATO, arsenic trioxide; ATRA, all-trans-retinoic acid; CR, complete response; DISH, differentiation induction subtraction hybridization; DMSO, dimethylsulfoxide; DTC, dacarbazine; ER, endoplasmic reticulum; GADD, growth arrest and DNA Damage inducible; HMBA, hexamethylenamine bisacetamide; IFN-β, interferon-beta; IL, interleukin; MDA, melanoma differentiation-associated; MEI, friend murine erythroleukemia cells; MEZ, mezerein; PCD, programmed cell death; PEG, progression elevated gene; PKC, protein kinase C; PMA, phorbol myristate acetate; PR, partial response; RaSH, rapid subtraction hybridization; RGP, radial growth phase; RSDD, reciprocal subtraction differentiation RNA display; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; TPA, 12-tetradecanoylphorbol-13-acetate; VGP, vertical growth phase

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INTRODUCTION

Overview of cancer and existing therapies

Cancer is a heterogeneous disease characterized by tumor cells that undergo continuous changes (both genetic and epigenetic) and undergo clonal expansion during the process of tumor cell evolution (Fisher 1984; Lowe 2004; Herceg 2007). A significant component of this process that characterizes the different forms of cancer is uncontrolled proliferation. Without treatment, specific primary tumors can acquire malignant potential that can culminate in death. Cancer is a multistep process caused by many factors including both external sources (exposure to carcinogens or infectious agents, diet) and internal features (defective inheritable traits, immune deficiencies), which can lead to the initiation and/or promotion of carcinogenesis (Fisher 1984; Cunha et al. 2002; Furuta et al. 2005; Goodrich 2006; Jones

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and Wells 2006; Herceg 2007).

Following the initial transformation of a normal cell into a tumor cell, many additional phenotypic and genetic changes occur concomitant with tumor progression that confer various growth promoting and anti-apoptotic advantages to the neoplastic cell (Fisher 1984; Cunha et al. 2002; Furuta et al. 2005). A hallmark of cancer is cellular immortality in which cancer cells possess unlimited growth potential within the host or in culture. This property contrasts the ability of normal cells to grow in culture to only 50-60 population doublings – a property known as the “Hayflick Limit” (Hayflick 1979). In addition to immortalization, transformed cells lose contact inhibition of growth, show decreased serum requirements, lose proper cell cycle control with failure to stop at cell cycle checkpoints, lose anchorage-dependent growth, show increased resistance to apoptosis, and can produce tumors in animals. As a consequence of these cellular changes that allow the transformed cell to grow beyond their normal environmental “stop” cues, some cancer cells no longer respond to cell-cell or cell-extracellular matrix interactions that normally serve to promote the differentiated cell state (Lowe et al. 2004; Furuta et al. 2005). Loss of certain micro-environmental cues can remove key signals required to maintain the differentiation phenotype (Bhowmick et al. 2004; Alini and Sporn 2007).

Cancer cells are frequently less differentiated than their progenitor normal cell type, a phenotype that correlates with the loss of specialized functions and increased capability to self-renew (Fisher and Grant 1985; Fisher et al. 1985a; Waxman et al. 1988; Fisher and Rowley 1991; Leszczyniecka et al. 2001). Cellular proliferation is a normal process that mostly occurs during development and in certain specialized tissue types, although upon terminal differentiation many cell types lose their proliferative capabilities. Following carcinogenesis cancer cells develop ways to overwhelm the proliferation checkpoints that are normally in place to restrain abnormal cellular growth. The concept of ‘differentiation therapy of cancer’ is based on the hypothesis that certain therapies can force cancer cells to again undergo terminal differentiation with an irreversible loss in proliferative capacity, which can in specific instances lead to programmed cell death, or PCD (Leszczyniecka et al. 2001; Jiang et al. 2004). This concept relies on the assumption that cellular factors (encoded by defined subset(s) of genes) that are required to re-program terminal differentiation are genomically intact but are either simply not expressed following cellular transformation or they are expressed at levels below the threshold required to elicit differentiation. In principle, forcing cancer cells to undergo terminal differentiation will halt proliferation and cancer progression, and in some cases PCD (apoptosis) will ensue.

Because cancer is the second leading cause of death in the United States and accounts for 1 out of every 4 deaths, a significant effort is being expended to understand how cancer develops with the expectation that this knowledge will lead to more effective means of treatments (Simmoff and Ross 2005). Current cancer therapies are comprised of surgery, chemotherapy or radiation, in which the latter two options evoke cytotoxicity to both cancer and normal host cells (Leszczyniecka et al. 2001; Kondagunta and Motzer 2006; Koon and Atkins 2007). As a result, most anti-cancer treatments cause adverse non-cancer cell-specific side effects. This review will discuss ‘differentiation therapy of cancer’ as a viable and potentially efficacious alternative approach to cytotoxic cancer therapies and will highlight important findings from the human melanoma differentiation model system with an emphasis on the translation of basic research findings to the clinical treatment of cancer patients.

DIFFERENTIATION THERAPY OF CANCER

Overview and rationale

Development and maturation of an organism results in fully differentiated cells that each perform various specialized processes. In specific tissues, such as skin, colon, blood, etc., differentiation is a continuous process resulting in the temporal development in functionally differentiated cells that are essential for maintenance of normal homeostasis. Under normal circumstances, most fully differentiated cells lose their proliferative ability and as such are “proliferation dead end” cells that exist to perform their defined function within the host. Upon transformation and tumor expansion, many neoplastic cells lose their differentiated phenotype and gain the ability to divide and proliferate with a potentially unlimited lifespan (Fisher 1984; Fisher and Grant 1985; Fisher et al. 1985a, 1985b; Waxman et al. 1988; Clark 1991; Fisher and Rowley 1991; Leszczyniecka et al. 2001). This observation has led to a fundamental premise on which the hypothesis for ‘differentiation therapy of cancer’ has been established: therapeutic agents can induce neoplastic cells to undergo terminal differentiation, and as such, these cells will lose their proliferative capacity and in some cases undergo PCD. ‘Differentiation therapy of cancer’ is an attractive proposal because it could potentially achieve more selective cancer target-cell specificity with less toxicity than current chemotherapy or radiation regimens (Leszczyniecka et al. 2001).

Reports on ‘differentiation therapy of cancer’

‘Differentiation therapy of cancer’ was first observed in leukemic cells when it was discovered that treatment with phorbol esters could restore a normal differentiation program (Sachs 1978; Huberman and Callaham 1979; Koefler et al. 1980). Phorbol myristate acetate (PMA, also known as TPA or 12-tetradecanoylphorbol-13-acetate) is a potent activator of Protein Kinase C (PKC), which results in activation of various signal transduction pathways and nuclear translocation of the PKC enzyme followed by trans-activation of genes involved in leukemic cell differentiation (Murphy and Norton 1993; Carey et al. 1996). Despite its ability to induce differentiation effects in certain cell types, PMA can also function as a tumor promoting agent and is often used to promote mouse skin carcinogenesis following initiation events that involve DNA damage (reviewed in Owens et al. 1999; Baird and Boutwell 1971; Bissey and Sirsat 1976; Angel and DiGiovanni 1999; Murphy et al. 2001).

At the center of these two opposite physiologic spectrums is the activation of PKC, though the effect of its activation in normal, initiated or malignant cells leads to different physiologic outcomes and suggests that other pathways may also control the physiological status and final fate of the cell.

Since these initial reports, other compounds have been discovered that are effective therapies against various neoplastic diseases. The most successful example of differentiation therapy of cancer is the treatment of acute promyelocytic leukemia (APL), which is a somewhat rare subtype of acute myeloid leukemia (AML) that has become the most curable AML in adults (Sanz et al. 2004; Zelent et al. 2005). Treatment of APL patients with all-trans-retinoic acid (ATRA) often results in the complete remission of disease and is also used in the treatment of head and neck cancer as well as neuroblastoma (Huang et al. 1989; Castaigne et al. 1990; Warrell et al. 1991). Combination of ATRA and anthracycline-based chemotherapy results in ~70-80% of patients being completely cured (Zelent et al. 2005). In vitro studies found that ATRA functions by inducing maturation and differentiation of leukemic cells in culture (Drach et al. 1993). Later studies found that ATRA could also induce PCD in leukemic cells in addition to inducing differentiation and growth inhibition (Gianni et al. 2000). More recent studies have shown that ATRA-induced...
Differentiation of leukemia cells can be enhanced by reducing the calcium accumulation in the endoplasmic reticulum (ER), further demonstrating that a detailed understanding of the molecular mechanisms involved in cancer cell differentiation can lead to the development of better therapeutics (Launay et al. 2002).

The high complete response (CR) rate of treated APL patients has, in turn, led to a relapse rate of ~10-30% for whom arsenic trioxide (ATO) is the therapy of choice (Soignet et al. 2001). ATO appears to function two-fold in APL by causing apoptosis at higher concentrations (1-2 μM) and by inducing differentiation at lower concentrations (0.1-0.5 μM) (Chen et al. 1997). However, recent investigations have shown no significant response to ATO as a front-line therapy for APL and substantiate its use as only a secondary therapeutic option (Sanz et al. 2006).

Other compounds can induce differentiation of leukemia cells with varying efficiencies. Bryostatin 1 inhibits growth of leukemia cells and also induces differentiation (Kraft et al. 1989; Asiedu et al. 1995). Like PMA, Bryostatin 1 binds to and activates PKC, but in contrast to PMA Bryostatin 1 does not function as a tumor-promoting agent (Hennings et al. 1987; Lewin et al. 1992). Preclinical studies with Bryostatin 1 have demonstrated anti-tumor activity in leukemia, lymphoma, and melanoma cells (Dale et al. 1989; Schuchter et al. 1991; Hornung et al. 1992). Clinical studies that combine Bryostatin 1 and other cytotoxic therapies in leukemia patients are encouraging and provide evidence to suggest that differentiation therapy can enhance other cytotoxic cancer therapies (Roberts et al. 2006).

Hexamethyamine bisacetamide (HMBA) can induce a monocytic differentiation program in myeloid leukemia cells (Haces et al. 1987), erythroid maturation in Friend murine erythroleukemia (MEL) cells (Gambari et al. 1979), and differentiation of human leukemia cells (Wu et al. 1991; Arcangeli et al. 1993). HMBA is a member of a group of poly-planar compounds and is thought to function by triggering cell cycle arrest in Go/G1 (Kiyokawa et al. 1993), activation of PKC (Melloni et al. 1987; Mallia et al. 1999), and modulation of intracellular calcium levels (Sparatore et al. 1995). Several clinical trials have been completed (Callery et al. 1986; Rowinsky et al. 1986; Egorin et al. 1987; Rowinsky et al. 1987; Young et al. 1988; Andreeff et al. 1992; Rowinsky et al. 1992) and though most have found no objective evidence of patient improvement, two reports document a positive response to HMBA therapy (Young et al. 1988; Andreeff et al. 1992).

The study by Young et al. involved a dose-escalation and pharmacokinetic study of 33 patients with advanced solid tumors, whereby objective anti-tumor effects (including transient regression of metastases or infiltrations) were observed in five patients although only one of these five was judged to have had clinical benefit from the response (Young et al. 1988). In contrast, Andreeff et al. chose 28 patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and observed a higher success rate of 12.1% that can be attributed by either compound (CR) or partial response (PR), though the authors report severe disease progression in seven other patients and note a “clearly suboptimal” therapeutic activity of HMBA alone (Andreeff et al. 1992). Overall, these studies suggest that HMBA therapy is not a robust therapeutic option on its own given the low number of objective responders in all of the studies taken together. Another hybrid polar compound like 22.1% that can induce various tumor cells to commit to terminal differentiation is dimethylsulfoxide (DMSO) (Collins et al. 1978; Huberman et al. 1979; Arcangeli et al. 1993; Dicevea et al. 1995). DMSO also prevents de-differentiation of normal hepatocytes in culture (Isom et al. 1985; Sato et al. 1988; Arterburn et al. 1995; Trompeter et al. 1999).

The cumulative reports on various agents that induce terminal differentiation of cancer cells is promising and warrants future investigation. The addition of differentiation-inducing agents to current cytotoxic therapies may provide an extra level of target cell specificity and potentially could enable lower dosing regimens of the cytotoxic component. A detailed molecular understanding behind both carcinogenesis and terminal differentiation of various cell lineages has and will enable better therapeutic options for cancer.

**DISCOVERY OF A NOVEL CYTOKINE FOR ANTI-CANCER THERAPY: INSIGHTS FROM THE STUDY OF HUMAN MELANOMA DIFFERENTIATION**

**Introduction to melanoma**

Melanoma has become an important public health issue due to its rising prevalence in Caucasian populations (Jemal et al. 2006). It is estimated that over the last 50 years, the incidence has risen steadily by around 6% each year leading to a 10-fold increase in frequency since the late 1950s. The increased incidence of melanoma is associated with increased sunlight exposure, particularly during early childhood years, though improved detection and documentation of tumor thickness over time could also contribute to the rise in melanoma incidence (Jemal et al. 2006). Currently, melanoma is the fifth and sixth most common cancer in men and women in the United States, respectively (Jemal et al. 2006). The development of malignant melanoma, with the exception of nodular-type melanoma, is perceived to be a multi-step process involving the conversion of a melanocyte into a nevus, a dysplastic nevus, a radial growth phase (RGP) primary melanoma, a vertical growth phase (VGP) primary melanoma and a metastatic melanoma (Herlyn 1990; Clark 1991; Miller and Mihm 2006). When the disease is confined to the epidermis and when it is thin (<1 mm) there is very little risk for metastatic spread and surgical resection results in a complete cure. However, with increasing thickness melanomas acquire metastatic potential and metastatic melanomas are universally fatal because of extreme resistance to adjuvant therapies such as radio- and/or chemotheraphy (Rigel and Carucci 2000; Jemal et al. 2005; Atallah and Flaherty 2006). Dacarbazine (DTC) is the current chemotherapy of choice for melanoma and has a modest response rate of 15-20% (Serrone et al. 2000; Atallah and Flaherty 2006). Early studies combining DTC with other cytotoxic therapies provided promising results. However, in recent clinical trials two separate regimens of either cisplatin, vinblastine, and DTIC or the Dartmouth regimen of DTIC, cisplatin, bischloroethylnitrosourea and tamoxifen did not increase overall survival compared to single DTIC therapy (Chapman et al. 2003; Serrone et al. 2000; Atallah and Flaherty 2006). These results highlight the need to develop more effective therapies and suggest that more non-toxic approaches, such as differentiation therapy, might be helpful as an alternative to harsh cytotoxic approaches.

Malignant melanoma is an ideal model to study the effects of cancer progression and differentiation because it epitomizes the classical process of step-wise tumor progression (Fisher et al. 1983). The study of 33 patients with advanced solid tumors, whereby objective anti-tumor effects (including transient regression of metastases or infiltrations) were observed in five patients although only one of these five was judged to have had clinical benefit from the response (Young et al. 1988). In contrast, Andreeff et al. chose 28 patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and observed a higher success rate of 12.1% that can be attributed by either compound (CR) or partial response (PR), though the authors report severe disease progression in seven other patients and note a “clearly suboptimal” therapeutic activity of HMBA alone (Andreeff et al. 1992). Overall, these studies suggest that HMBA therapy is not a robust therapeutic option on its own given the low number of objective responders in all of the studies taken together. Another hybrid polar compound like 22.1% that can induce various tumor cells to commit to terminal differentiation is dimethylsulfoxide (DMSO) (Collins et al. 1978; Huberman et al. 1979; Arcangeli et al. 1993; Dicevea et al. 1995). DMSO also prevents de-differentiation of normal hepatocytes in culture (Isom et al. 1985; Sato et al. 1988; Arterburn et al. 1995; Trompeter et al. 1999).

The cumulative reports on various agents that induce terminal differentiation of cancer cells is promising and warrants future investigation. The addition of differentiation-inducing agents to current cytotoxic therapies may provide an extra level of target cell specificity and potentially could enable lower dosing regimens of the cytotoxic component. A detailed molecular understanding behind both carcinogenesis and terminal differentiation of various cell lineages has and will enable better therapeutic options for cancer.
General methods to identify differentiation-associated genes

To comprehend the mechanism of terminal cell differentiation in HO-1 human melanoma cells, multiple molecular approaches are being used to define the complete spectrum of gene expression changes associated with and potentially mediating differentiation (Jiang and Fisher 1993; Jiang et al. 2003). Classical subtraction hybridization processes and improved modifications of this scheme, such as Differentiation Induction Subtraction Hybridization (DISH) (Fig. 1), Reciprocal Subtraction Differentiation RNA Display (RSDD) and Rapid Subtraction Hybridization (RaSH), and cDNA microarrays are being used to identify genes that are selectively upregulated by IFN-β + MEZ treatment of HO-1 cells compared to untreated cells (Fig. 1) (Jiang and Fisher 1993; Jiang et al. 1994, 1995a, 1995b, 1996; Kang et al. 1998; Huang et al. 1999a, 1999b; Jiang et al. 2000). Using this methodology a subset of melanoma differentiation associated (mda) genes (both known and unknown) that are upregulated during terminal differentiation were cloned and identified (Fig. 1). Some of the key mda genes that were isolated and cloned by this method include mda-6, which is the ubiquitously expressed kinase inhibitor p21 (Jiang and Fisher 1993; Jiang et al. 1994, 1995b), mda-5, a putative RNA helicase functioning as the first sensor of RNA virus infection thus playing a key role in regulating innate immunity and promoting apoptosis (Kang et al. 2002; Lin et al. 2006), mda-9/syntenin, a PDZ-containing adaptor protein that is a positive regulator of metastasis (Sarkar et al. 2004a; Boukerche et al. 2005, 2006), human polynucleotide phosphorylase, a 3′-5′ exonuclease associated with cellular senescence, age-associated inflammation and response to interferon (Leszcyniecka et al. 2002, 2003; Sarkar et al. 2003, 2004b, 2005a, 2005b; Sarkar and Fisher 2006a, 2006b, 2006c) and mda-7/IL-24, a novel member of the IL-10 gene family that induces cell death in many types of cancer but not normal cells (Fig. 2) (Jiang et al. 1995a, 1996; Su et al. 1998; Madireddi et al. 2000; Fisher 2005; Gupta et al. 2006a; Sarkar et al. 2006a). Very interestingly, each of the mda genes that have been characterized so far plays a pivotal role in various aspects of cellular physiology, and as such clearly demonstrates the power of the DISH approach (Fig. 1).

A novel anti-cancer specific cytokine: mda-7/IL-24

Upon initial analysis of the mda genes identified by the DISH approach, expression of mda-7 mRNA was found to be upregulated following terminal differentiation of HO-1 human melanoma cells (Jiang and Fisher 1993; Jiang et al. 1995a). Subsequent investigation of mda-7 transcripts during melanoma progression showed its expression in normal melanocytes with progressively decreased expression of mRNA and protein during the progression from normal melanocyte to metastatic melanoma (Jiang et al. 1995a; Ekmićcioglu et al. 2001; Ellerhorst et al. 2002; Lebedeva et al. 2002). Sequence analysis of mda-7 revealed a signature motif characteristic of IL-10 family members and its genomic location on chromosome 1q32 is within a region that contains a cluster of other IL-10 family cytokine genes, namely IL-19, IL-20 and IL-26 (Jiang et al. 1995a; Huang et al. 2001; Pestka et al. 2004). Based on mda-7/IL-24’s genomic localization, functional studies demonstrating that mda-7/IL-24 exhibits immunostimulatory activity, expression in specific cells in the immune system, secretory property and homology to other interleukin family members, MDA-7 was renamed to interleukin-24 (IL-24) (Huang et al. 2001; Caudell et al. 2002; Sauane et al. 2003; Pestka et al. 2004).

MDA-7/IL-24 is secreted by a variety of cells types of the immune system as well as melanocytes and can bind to functional heterodimeric receptors IL-20R1/IL-20R2 and IL-22R1/IL-20R2 on target cells, which in turn leads to STAT activation (Dumoutier et al. 2001; Wang et al. 2002). Recently Kunz et al. (2006) reported that mda-7/IL-24, along with other IL-10 family members IL-19 and IL-20, is...
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also produced by and acts upon keratinocytes in vitro and is produced primarily in vivo by inflamed tissues. These observations suggest that MDA-7/IL-24 normally functions in a non-classical interleukin, and as such is a member of a unique subset of mediators within the IL-10 family (Pestka et al. 2004; Kunz et al. 2006; Sarkar et al. 2006a).

The observation that mda-7/IL-24 expression inversely correlates with melanoma progression and growth potential led to the hypothesis that forced expression of mda-7/IL-24 would lead to growth inhibition (Jiang et al. 1995a, 1996). This hypothesis was proven true not only in melanoma cells, but also in many other cancer cell types (Jiang et al. 1995a, 1996). Supra-physiological expression of mda-7/IL-24 not only inhibits cancer cell growth, but also induces apoptosis in cancer cells while leaving normal cell growth and viability unaffected (Jiang et al. 1996; Su et al. 1998). In fact, the growth and viability of a substantial array of normal cells are not affected by expression of mda-7/IL-24 while the corresponding tumor cells undergo growth inhibition and apoptosis following forced mda-7/IL-24 expression (Fisher 2005; Gupta et al. 2006a; Lebedeva et al. 2007).

Extensive studies have been carried out to elucidate the mechanism whereby MDA-7/IL-24 can selectively induce apoptosis in cancer cells but not normal cells (Fig. 2) (reviewed in Fisher 2005; Gupta et al. 2006a; Sarkar et al. 2006a; Lebedeva et al. 2007). Intracellular localization studies have shown that MDA-7/IL-24 localizes to the endoplasmic reticulum (ER) and Golgi compartments of both normal and cancer cells, however MDA-7/IL-24 selectively induces an ER stress response in cancer cells and interacts with the ER chaperone protein BiP/GRP78, which is crucial in mediating MDA-7/IL-24 action (Sauane et al. 2002; Su et al. 2003). In addition, the treatment of cancer cells with MDA-7/IL-24 changes the ratio of pro-apoptotic (Bax/Bak) to anti-apoptotic (Bcl-2/Bcl-x_L) proteins, further shifting the cancer cell physiology towards PCD (Su et al. 1998; Lebedeva et al. 2002) (Fig. 2).

In addition to killing cancer cells following translation of its mRNA, MDA-7/IL-24 can also be secreted into the extracellular milieu. Importantly, secreted MDA-7/IL-24 can also induce cell death in distant cancer cells that express its cognate receptor thereby demonstrating potent “bystander” anti-tumor activity (Su et al. 2001a; Sarkar et al. 2005b; Su et al. 2005b). The “bystander” anti-tumor property of MDA-7/IL-24 acts to amplify its efficacy at inducing cell death of cancer cells by killing not only the cancer cells that are directly targeted to receive and express the mda-7/IL-24
gene, but also by killing surrounding cancer cells after secretion of MDA-7/IL-24 from the targeted cells (Su et al. 2001a; Chada et al. 2004; Sarkar et al. 2005b; Su et al. 2005a; Sarkar et al. 2006c; Zheng et al. 2006). These observations have fueled the idea that mda-7/IL-24 is an ideal candidate for use in gene therapy of cancer (Fisher et al. 2003; Fisher 2005; Lebedeva et al. 2005; Fisher et al. 2006; Gupta et al. 2006a; Sarkar et al. 2006a; Lebedeva et al. 2007).

The generation of a replication-deficient adenovirus expressing mda-7/IL-24 (Ad.mda-7) has permitted in vivo studies where Ad.mda-7 treatment led to the inhibition of tumor growth in mice with xenografts of human cancers including breast, colon, lung, brain, pancreatic, melanoma and prostate cancer (Su et al. 1998, 2001b; Saeki et al. 2002; Yacoub et al. 2004; Sarkar et al. 2005b; Zhao et al. 2005; Lebedeva et al. 2006; Sarkar et al. 2006c; Zhao et al. 2006). In addition, the “bystander” anti-tumor activity of MDA-7/IL-24 was observed in vivo whereby animals given tumor xenografts in both flanks were injected with Ad.mda-7 in only the left flank, but inhibition of tumor growth was observed in both the left flank tumor that was treated as well as in the distal right tumor (Sarkar et al. 2005b, 2006c). To improve the delivery and efficacy of mda-7/IL-24 as a gene therapeutic for cancer, Sarkar et al. (2005b, 2006c) developed a novel ‘Cancer Terminator Virus’ in which targeted viral replication in cancer cells was controlled by the cancer-specific progression elevated gene-3 (PEG-3) (Su et al. 1997) promoter (Su et al. 2000, 2001b, 2005c, 2006c) resulting in production of mda-7/IL-24 as a function of virus replication uniquely in cancer cells (Fig. 3). This novel virus, Ad.PEG-E1A-mda-7 (a ‘Cancer Terminator Virus’), produced cures in animals containing breast or prostate carcinomas or melanomas growing on both flanks in which tumors only on the left flank were injected with virus, which was not observed in animals receiving non-replicating Ad.mda-7 (Sarkar et al. 2005b, 2006c; and unpublished data). The success of the animal studies with Ad.mda-7 and Ad.PEG-E1A-mda-7 provide compelling evidence to further analyze its use as a therapeutic option in human cancer patients.

**Clinical trial with adenovirus-delivered mda-7/IL-24 (Ad.mda-7; INGN 241)**

A phase I clinical trial was performed to assess the safety of Ad.mda-7 (INGN 241) by intratumoral injections in 22 human patients with melanoma and resectable solid tumors in a dose-escalation study (Fisher et al. 2003; Cunningham et al. 2005; Lebedeva et al. 2005; Tong et al. 2005; Fisher et al. 2006; Lebedeva et al. 2007). The direct injection of Ad.mda-7 was generally well tolerated and successful gene transfer was observed by the presence of transgene DNA and mRNA in all tumors and the highest levels of mda-7/IL-24 expression at/near the site of injection. However, MDA-7/IL-24 protein expression was detectable in the periphery of injected lesions beyond the area of DNA spread, supporting the in vitro “bystander” anti-tumor observations that MDA-7/IL-24 can diffuse from transduced cells. Immuno-nostaining of MDA-7/IL24 protein correlated with induction of apoptosis in tumor cells and INGN 241 treatment also induced increased numbers of CD3+CD8+ T cells and transiently increased serum levels of TGF-β cytokines such as IL-6, IL-10, and TNF-α (Tong et al. 2005).

The clinical response to Ad.mda-7 was encouraging with four of nine (44%) injected lesions demonstrating objective response [complete response (CR) or partial response (PR)] in the patient cohort that experienced the longest dosing regimen involving multiple injections with Ad.mda-7. The two cohort 8 patients that showed clinically significant responses were both melanoma patients. One of these two patients had more than 10 discrete lesions and the initial site of treatment was a supracervical node measuring 2 × 2 cm at baseline. Following the final sixth injection of INGN 241, the lesion size clearly decreased and regression continued over the next two weeks until there was no clinical evidence of disease at that site (Fisher et al. 2006). Overall the Phase I clinical trial demonstrated that intra-tumoral injection of Ad.mda-7 (INGN 241) induced apoptosis in a large volume of tumors and stimulated activation of the patients’ immune response while clinically significant responses were primarily seen following repeat injections. As such, this initial foray into the clinic has provided promising results warranting future pursuit of the use of mda-7/IL-24 as a novel anti-cancer therapy. The ability of mda-7/IL-24 to promote the anti-cancer properties of chemotherapy, radiation and monoclonal antibody in vitro and in vivo in animal models suggest that a combinatorial approach could be particularly effective in treating diverse cancers (Su et al. 2003; Bocangel et al. 2006; Chada et al. 2006; Emdad et al. 2006; Lebedeva et al. 2006; Su et al. 2006; Emdad et al. 2007; Inoue et al. 2007). Additionally, studies in prostate and lung cancers indicate that the combination of mda-7/IL-24 with radiation or gefitinib, respectively, can reverse resistance observed in specific tumors when treated singly with either therapy (Su et al. 2006; Emdad et al. 2007).
CONCLUDING REMARKS

A major challenge facing the medical and scientific community is to develop safe, effective and enduring therapies for patients with cancer. In this context, developing molecular target-based treatment paradigms offers significant potential to achieve these objectives. This type of approach must take into account the fundamental characteristics of cancer, including recurrent multiple genetic mutations mediating unrestrained cell growth and frequent resistance to apoptosis-inducing agents, thereby drastically decreasing the probability that any single therapeutic will be efficacious for all types of cancer. Resistance to current treatments such as surgery, chemo- and radiation-therapy, has prompted creative ideas for alternative therapeutic options. “Differentiation therapy of cancer”, whereby therapeutic agents induce neoplastic cells to undergo terminal differentiation and lose their proliferative capacity (and in some cases undergo programmed cell death), is an attractive alternative to conventional cytotoxic therapies that often elicit significant non-cancer cell-specific toxicity and harsh side effects in patients (Leszynecka et al. 2001).

The current success of differentiation therapy of APL has led to its classification as the most curable AML in adults (Sanz 2004; Zeleni 2005). Other in vitro studies have led to important findings that, in turn, have led to improved anti-cancer therapies as a result of increased understanding of cancer cell biology. Investigations that were initiated to identify genes involved in the terminal differentiation of human melanoma cells not only led to the identification of both known and unknown mda genes that are key regulators of a multitude of cellular processes, but also to the discovery of the novel anti-cancer cytokine mda-7/IL-24. Based on the substantial in vitro, in vivo, and clinical results, mda-7/IL-24 appears to be a promising novel anti-cancer therapeutic and, as such, represents a success story stemming from the pursuit of a better understanding of mechanisms underlying terminal differentiation and irreversible loss of growth potential of cancer cells (Fisher 2005). These studies and the steady scientific progression reflects a fundamental mantra of basic/medical science, from ‘bench to bedside’, that is already starting to reap significant rewards.

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