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The development of MDA-7/IL-24 as a cancer therapeutic

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ABSTRACT

The cytokine melanoma differentiation associated gene 7 (*mda*-7) was identified by subtractive hybridization as a protein whose expression increased during the induction of terminal differentiation, and that was either not expressed or was present at low levels in tumor cells compared to non-transformed cells. Based on conserved structure, chromosomal location and cytokine-like properties, MDA-7, was classified as a member of the interleukin (IL)-10 gene family and designated as MDA-7/IL-24. Multiple studies have demonstrated that expression of MDA-7/IL-24 in a wide variety of tumor cell types, but not in corresponding equivalent non-transformed cells, causes their growth arrest and rapid cell death. In addition, MDA-7/IL-24 has been noted to radiosensitize tumor cells which in part is due to the generation of reactive oxygen species (ROS) and ceramide that cause endoplasmic reticulum stress and suppress protein translation. Phase I clinical trial data has shown that a recombinant adenovirus expressing MDA-7/IL-24 (Ad. *mda*-7 (INGN-241)) was safe and had measurable tumoricidal effects in over 40% of patients, strongly arguing that MDA-7/IL-24 could have significant therapeutic value. This review describes what is presently known about the impact of MDA-7/IL-24 on tumor cell biology and its potential therapeutic applications.

Contents

Abbreviations: ERK, extracellular regulated kinase; MEK, mitogen activated extracellular regulated kinase; JNK, c-Jun NH_2-terminal kinase; PI3K, phosphatidyl inositol 3 kinase; MDA-7, melanoma differentiation associated gene 7; IL-24, interleukin-24; PERK, protein kinase R-like endoplasmic reticulum kinase; MAPK, mitogen activated protein kinase; ca, constitutively active; dn, dominant negative; EGFR, epidermal growth factor receptor; PTEN, phosphatase and tensin homologue on chromosome ten.

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1. Background to MDA-7/IL-24

MDA-7/IL-24 was discovered using a subtraction hybridization approach by exposing melanoma cells to the terminal differentiation-inducing agents interferon beta and mezerein (Jiang & Fisher, 1993; Jiang et al., 1993, 1995). Based on a conserved amino acid signature sequence, chromosomal location and cytokine-like properties, *mda*-7, has been classified as a member of the expanding interleukin (IL)-10 gene family, which includes IL-10, IL-19, IL-20, IL-22 and IL-26, and has been designated as *mda*-7/IL-24 (Jiang et al., 1995; Ekmekcioglu et al., 2001; Huang et al., 2001; Ellerhorst et al., 2002; Wolk et al., 2002; Pestka et al., 2004). MDA-7/IL-24 protein expression is decreased in advanced melanomas, with nearly undetectable levels in metastatic disease, in general agreement with this gene product being classified as a tumor suppressor (Jiang et al., 1995; Jiang et al., 1996; Huang et al., 2001; Wolk et al., 2002). Other published studies over the last 15 years have demonstrated that enforced expression of MDA-7/IL-24, either by transfection of a plasmid containing the cDNA for *mda*-7/IL-24 or by use of a recombinant adenovirus to deliver the gene, Ad.*mda*-7, rapidly inhibits the growth of a broad-spectrum of cancer cells, resulting in tumor cell death within 24–48 h (Jiang et al., 1993; Jiang & Fisher, 1993; Jiang et al., 1995; Su et al., 1998; Huang et al., 2001; Ekmekcioglu et al., 2001; Wolk et al., 2002; Ellerhorst et al., 2002; Kotenko, 2002; Caudell et al., 2002; Pestka et al., 2003, 2004; Fisher, 2005; Lebedeva et al., 2005, 2007b). When expressed, MDA-7/IL-24 is secreted from cells, as would be expected for a cytokine. Of considerable note, when MDA-7/IL-24 was over-expressed in non-transformed cells little change was observed in either cell growth or cell viability (e.g., Jiang et al., 1996).

Initial studies using mammalian cell-synthesized MDA-7/IL-24 protein; a protein that is a dimer and glycosylated, demonstrated that purified MDA-7/IL-24 interacted with two type II cytokine heterodimeric receptor complexes: IL-20R1/IL-20R2 (type 1 IL-20R) and IL-22R1/IL-20R2 (type 2 IL-20R) (Parrish-Novak et al., 2002). In one of the first of these studies, non-transformed BHK cells stably transfected with IL-20 and IL-22 receptors were treated with MDA-7/IL-24; at low pM concentrations of MDA-7/IL-24 (<100 pM) growth was promoted whereas at higher concentrations (>100 pM) it inhibited cell proliferation. In transfected cells, MDA-7/IL-24 activated multiple STAT transcription factors. However, in ovarian carcinoma cells, which express endogenous IL-20 receptor complexes, it was noted that MDA-7/IL-24 at low nM concentrations promoted growth inhibition without altering STAT transcription factor phosphorylation/function (Parrish-Novak et al., 2002; Chada et al., 2004a,b). Other studies have demonstrated using tumor cells, which lack STAT1 or STAT3 function or with blocked Janus kinase function that STAT pathway signaling is not required for MDA-7/IL-24-induced growth arrest or tumor cell killing (Sauane et al., 2003).

More recently, experiments indicate a difference in the cell signaling and cell killing properties of bacterial synthesized unglycosylated and monomeric GST-MDA-7/IL-24 and mammalian cell-synthesized glycosylated dimeric MDA-7/IL-24 with FLAG or $(His)_6$ tags to assist in isolation and purification. In multiple studies using a wide variety of transformed cell lines, GST-MDA-7/IL-24 has been noted to promote cell growth arrest and apoptosis in a transformed cell-specific fashion and has been noted to cause these effects independently of expression of IL-20 receptors, in a similar manner to Ad.*mda*-7 (Sauane et al., 2004a; Lebedeva et al., 2007b; Emdad et al., 2009; and references therein). This would suggest that cancer cells take up GST-MDA-7/IL-24 in an interleukin receptor-independent fashion (Sauane et al., 2004a). In contrast to GST-MDA-7/IL-24 and Ad.*mda*-7, purified MDA-7/IL-24, synthesized in mammalian cells, does not appear to have any biologic effect on cells lacking expression of IL-20 receptor complexes (Sauane et al., 2008). Of note however, and in a similar manner to GST-MDA-7/IL-24 and Ad.*mda*-7, in cells where IL-20 receptor complexes were expressed, mammalian synthesized MDA-7/IL-24-induced cell killing was independent of STAT transcription factor activation. For example, in A549 human lung carcinoma cells, which lack expression of the IL-20 receptor complexes, extracellular treatment with mammalian cell-synthesized MDA-7/IL-24 results in no biologic effect on cell growth/viability, whereas treatment with GST-MDA-7/IL-24, or viral infection with Ad.*mda*-7 or Ad.*mda*-7 signal peptide null (SP-), which expressed a non-secreted form of MDA-7/IL-24 or transfection of a plasmid to express MDA-7/IL-24 all results in tumor cell growth arrest and cell death (Nishikawa et al., 2004; Sauane et al., 2004a,b; Pataer et al., 2007). Furthermore, although it has been noted that MDA-7/IL-24, IL-20, and IL-19 all activated STAT transcription factors in IL-20 receptor expressing cancer cells, only MDA-7/IL-24 has the ability to cause cell death (Chada et al., 2006). Collectively, this data argues that the direct tumoricidal effects of MDA-7/IL-24 when expressed intracellularly are independent of IL-20 receptor complex signaling and instead are dependent on an additional biological property of MDA-7/IL-24.

2. MDA-7/IL-24 and apoptosis

The pathways by which Ad.*mda*-7 (or: transfection with a cDNA to express MDA-7/IL-24; treatment with bacterial synthesized GST-MDA-7/IL-24 or eukaryotic cell generated $_{His6}$-MDA-7/IL-24) enhances apoptosis in tumor cells are still not completely understood, however, over the last 7 years a large amount of evidence from multiple studies has demonstrated the involvement of proteins important in the regulation of endoplasmic reticulum (ER) stress and mitochondrial integrity (Lebedeva et al., 2003a,b; Gupta et al., 2006a; Lebedeva et al., 2007a; Yacoub et al., 2008a; Park et al., 2009; Yacoub et al., 2010a; Hamed et al., 2010; Yacoub et al., 2010b) (Fig. 1). Some studies have argued that MDA-7/IL-24 promoted activation of the double stranded RNA-activated kinase, Protein Kinase R (PKR), which was correlated with enhanced eIF2 alpha phosphorylation and MDA-7/IL-24-stimulated cell death. In this study PKR null fibroblasts were resistant to IL-24-induced apoptosis, although subsequent studies from the same group have argued that PKR does not always play a role in the lethal effects of MDA-7/IL-24 (Pataer et al., 2002, 2005).

In studies from our laboratories we noted that MDA-7/IL-24 binds to the HSP70 family chaperone BiP/GRP78. Binding of MDA-7/IL-24 to BiP/GRP78 inactivates the chaperone function of the protein promoting its dissociation from PKR-like endoplasmic reticulum kinase (PERK) (Gupta et al., 2006b). Over-expression of BiP/GRP78 suppresses MDA-7/IL-24-induced toxicity (Yacoub et al., 2008a, 2010a). Dissociation of BiP/GRP78 from PERK promotes PERK trans-phosphorylation and activation, and subsequently the phosphorylation and activation of eIF2 alpha. The phosphorylation of eIF2 alpha in turn leads to the global suppression of protein translation which, with respect to its tumor cell killing properties, results in reduced expression of anti-apoptotic proteins that have short half lives such as MCL-1, BCL-XL and c-FLIP-s (Fels & Koumenis, 2006; Fritsch et al., 2007; Raven & Koromilas, 2008). Indeed, some of the earliest correlative observations regarding MDA-7/IL-24 toxicity were that the cytokine decreased expression of BCL-XL and enhanced expression of toxic BH3 domain proteins such as BAX and BAK (Su et al.,