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The Nm23-H1 metastasis suppressor as a translational target

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ABSTRACT

Nm23 was the first of what has become a field of over 20 known metastasis suppressor genes (MSGs). Since the discovery of Nm23 in 1988, a variety of mechanisms have been attributed to its activity, including a histidine kinase activity, binding of other proteins to regulate metastatic formation, and altered gene expression downstream of Nm23. Here, we will review current efforts to translate the previous work done on this MSG into the clinic, including high-dose medroxyprogesterone acetate (MPA), which has been shown to upregulate Nm23 expression. In addition, we will detail a new potential target downstream of Nm23. LPA1 is one of a group of known cell surface receptors for lysophosphatidic acid (LPA), which has been shown to be inversely correlated with Nm23 expression. A specific LPA1 antagonist could conceivably mimic the effects of Nm23 by downregulating the activity of the LPA1 pathway, which would be of considerable interest for potential clinical use.

1. Introduction

Metastasis, the process in which cancer cells leave the primary tumour and spread to other tissues and organs to form new tumours, is the most common cause of cancer mortality. In order to increase the survival of patients, it is necessary to develop more effective methods for treating established metastatic disease and, ultimately, to develop methods that prevent the establishment of metastasis altogether. The spread of single cancer cells into the vasculature or to a secondary site does not by itself constitute metastasis.[1] Rather, the development of clinically detectable metastases requires that these cells complete a series of well-defined steps. To begin with, tumour cells must break away from the primary tumour, migrate through the extracellular matrix, and intravasate into the circulatory or lymphatic systems. There, the cells must resist anoikis, survive transport, and evade immune detection before arresting or adhering in major capillary beds. Although extravasation may or may not ensue, proliferation and colonisation of these cells in the secondary parenchyma follow. These steps are together referred to as the metastatic cascade and completion of each step is necessary for metastatic development.[2] At the time of diagnosis, it is believed that only 6% of breast cancer patients will present with clinically detectable metastatic disease. Thus, the metastatic cascade is incomplete in the majority of patients, providing a valuable window of opportunity for clinicians to exploit. It is within this window, prior to colonisation and the growth of large metastasis, that the possibility exists to significantly impact patient survival.

Targets of increasing interest for this are the metastasis suppressor genes (MSGs). These are genes that, by definition, do not affect the growth of the primary tumour, but significantly inhibit the process of metastasis and reduce the formation of metastatic foci. Twenty-three such genes have now been described in the literature and collectively make up the metastasis suppressor gene family.[3] These genes, path-

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ways and downstream molecules have become the focus of significant research during the past decade.

2. Nm23-H1

The first metastasis suppressor gene, *nm23*, was identified in 1988 by differential colony hybridisation.[4] It was first discovered by injecting seven cell lines derived from a single K-1735 murine melanoma into syngeneic and nude mice. All the cell lines formed primary tumours, but varied widely in the number of metastases present at the experimental endpoint. Differential gene expression studies identified a candidate cDNA, *nm23*, the expression of which was downregulated in five highly metastatic cell lines as compared with two related, less metastatic cell lines. Ectopic expression of *nm23* suppressed metastasis without altering primary tumour growth. These findings provided the evidence that the expression of specific genes is reduced in tumour cells that have acquired the ability to form metastases and the reintroduction of such genes can suppress the metastatic phenotype.

Therapeutic approaches to restore the anti-metastatic function of *Nm23-H1* (the first in the family of eight *Nm23* human homologues) have been attempted using a range of different strategies including *Nm23-H1* promoter activation by medroxyprogesterone acetate (MPA) treatment, activation of downstream gene targets, and gene therapy, all of which will be reviewed later in this article.

3. Activities of Nm23-H1

Nm23-H1 has a variety of validated molecular activities, with at least some playing important roles in regulating its ability to inhibit metastasis.[3] The first of these activities is that of a nucleoside diphosphate kinase (NDPK) that reversibly catalyses the phosphorylation of nucleoside 5′-diphosphates to triphosphates via autophosphorylation of an internal histidine (H118). Although this activity is well characterised, there have been no reported correlations between the NDPK activity of Nm23-H1 and its metastasis-suppressive effects.

The phosphohistidine of Nm23-H1 also participates as a histidine protein kinase. Histidine kinases are best studied in bacteria and lower eukaryotes, where they function in two-component signal transduction systems, transferring a phosphate from a sensor protein to a conserved aspartate residue on a response-regulator protein in reaction to an external stimulus.[5,6] In mammalian cells histidine kinases are poorly studied because of the acid lability of the histidine phosphate, which renders it undetectable under many common experimental conditions. Despite this, there are three known substrates for Nm23-H1 as a histidine kinase.

The first is ATP-citrate lyase, the primary enzyme that catalyses the production of cytosolic acetyl-CoA.[7] Acetyl-CoA is used in multiple biosynthetic pathways including lipogenesis and cholesterogenesis. Nm23-H1 phosphorylates a histidine residue at the catalytic site of ATP-citrate lyase, although the effects of this phosphotransfer remain unknown.[7] The second substrate of the histidine kinase activity of Nm23-H1 is aldolase C, an enzyme found primarily in the brain that is critical in glycolysis.[8] Nm23-H1 phosphorylates aldolase C on aspartate 319, although once again the biological ramifications of this phosphorylation are not understood. The last known histidine kinase substrate for Nm23-H1 is the kinase suppressor of Ras (KSR). KSR is a scaffold protein for the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway.[9,10] To date no clinically relevant therapeutic targets have been developed from these known substrates of Nm23-H1.

4. Medroxyprogesterone acetate

Recently, compounds capable of upregulating MSGs in cancer cells have been reported. One of these is MPA, which has been shown to elevate Nm23 expression at high doses in MDA-MB-231 and MDA-MB-435 human breast carcinoma cell lines. MPA was identified by analysis of the *Nm23-H1* promoter, which revealed a 248-bp region regulating reporter activity.[11] This region contained a cassette for transcription factor binding sites present in the mouse mammary tumour virus-long terminal repeat. This cassette of transcription factors is regulated by glucocorticoid response elements, presenting a potential target for the upregulation of Nm23. MPA binds progesterone, androgen and glucocorticoid receptors. At high doses, MPA was found to upregulate Nm23 expression in progesterone receptor-negative, glucocorticoid receptor-positive metastatic MDA-MB-231 and MDA-MB-435 breast carcinoma cell lines *in vitro*.[11] Exposure to high-dose MPA led to decreased anchorage-independent colonisation, which was abrogated once cells were transfected with antisense *Nm23-H1*, proving that MPA was functioning by elevating Nm23 levels.

To test the hypothesis that the elevation of Nm23 by MPA would lead to decreased metastatic colonisation *in vivo*, we set up the following experiment. MDA-MB-231 breast cancer cells were injected into immunocompromised mice and 4 weeks later, at which point micrometastasis was present, the mice were randomised to vehicle or MPA. MPA dosing reduced the number of gross pulmonary metastases in these mice from 33% to 46% and reduced both incidence and size of the metastases that formed. Limited side-effects recorded, including weight gain, while no change in bone density, mammary tissue histology or the lean to fat tissue ratio were found.[12]

Based on these data, as well as on previous Nm23-H1 studies, a Phase II trial has been initiated to test this new potential application of high-dose MPA (Kathy D. Miller, PI, Indiana University). The primary objective is to determine the clinical benefit of MPA monotherapy and MPA + low-dose oral cyclophosphamide and methotrexate ('metronomic therapy', IdoCM) in postmenopausal patients with refractory hormone receptor-negative metastatic breast cancer. A starting daily oral dose of 1 g MPA will be administered and increased to 1.5 g if serum concentrations are <50 ng/ml. In a second cohort, 'metronomic' IdoCM will be administered based on its reported anti-angiogenic activity.[13] Preclinical studies suggested greater activity when metronomic chemotherapy is combined with a second anti-angiogenic agent.[14,15]