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Review

Thinking outside the box: Using metastasis suppressors as molecular tools

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ABSTRACT

Metastasis, the process in which tumor cells move from a primary tumor through the circulation, lodge, and grow in distant locations, is a significant contributor to cancer patient morbidity and mortality, yet remains poorly understood. The molecular processes regulating tumorigenicity and metastasis are distinguishable, suggesting that it is possible to design therapeutic interventions to specifically control metastasis formation. Metastasis suppressors, which specifically regulate metastasis, are being used in "reverse genetics" approaches to discover the phenotypic alterations caused by modulating their levels and/or activity. This strategy is allowing the identification of tumor–host interactions that are crucial for efficient colonization and their disruption can be targeted to suppress metastases formation. In this review we discuss studies addressing invasion and migration, key functions for both early and late in the metastatic process. Metastasis suppressor functions, which modulate lodging and subsequent colonization of the secondary site, are also described. In sum this review focuses on metastasis suppressors that have yielded insight into mechanisms controlling metastasis formation. These serve as platform for out of the box thinking which will enable the discovery of new paradigms in metastasis research.

1. Introduction

Metastasis, the process in which tumor cells move from a primary tumor through the circulation, lodge, and grow in distant locations, is a significant contributor to cancer patient morbidity and mortality, yet remains poorly understood. The molecular processes regulating tumorigenicity and metastasis are distinguishable, suggesting that it is possible to design therapeutic interventions to specifically control metastasis formation. Unfortunately, once metastases can be detected clinically, treatments available to patients are mainly palliative and in most instances the chance of long-term survival is rare. Improved strategies to prevent and control the development of metastases require a robust understanding of tumor–host interactions that dictate the biologic fate of cancer cells during their transit from the primary tumor to secondary sites. During the process of transformation and subsequent progression to a malignant phenotype, both genetic and epigenetic alterations alter a cell ability to perceive and respond to signals that regulate normal tissue homeostasis. Metastasis is an inefficient process, as the majority of cancer cells escaping from the primary tumor or lodging at secondary sites will never give rise to clinically detectable metastases [1]. A minority of tumor cells accrue the genetic and epigenetic alterations required to disseminate from the primary tumor, survive insults from the immune system and biophysical forces, and finally lodge and grow in distant tissues.

After the identification of the first metastasis suppressor Nm23 and the establishment of a novel concept that a single protein could inhibit the formation and development of metastases without affecting the primary tumor [2], the field of metastasis suppressor research experienced three distinct phases (Fig. 1). During the daring hypothesis phase (1985–1995) investigators used laborious forward genetics approaches to identify the gene(s) responsible for the metastasis suppression phenotype. The challenges inherent in combining molecular techniques such as positional cloning with classical in vivo metastasis assays resulted in a seven year gap between the discovery of nm23 the report of KAI1, the second metastasis suppressor reported in the literature [3].

From 1995 to 2005 a number of metastasis suppressors were reported. During this "genes and functions" phase, investigators mainly focused on identifying the biochemical and cellular function(s) of novel metastasis suppressors like Nm23 and KISS1. For proteins with known functions such as mitogen-activated protein (MAP) kinase kinase 4 (MKK4) or Src Suppressed C Kinase Substrate (SSeCKS) investigators were able to conduct innovative mechanism-based studies to determine signaling events and pathways that regulate metastasis formation. Interestingly, the prevailing view during this period was that invasion was the rate-

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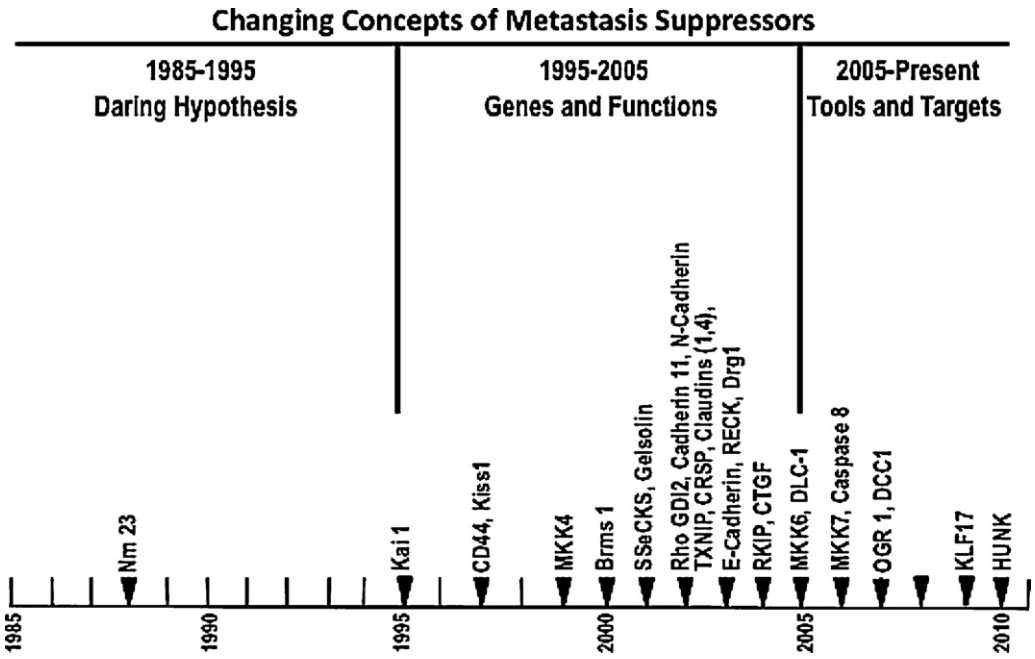

Fig. 1. Phases of development of the field of metastasis suppressor research.

limiting step for metastases formation. Careful biological studies using metastasis suppressors and other approaches used to study metastatic colonization showed that this is not the case. It is now widely accepted that metastasis suppressors can exert a significant growth control at secondary sites. These studies identified control of disseminated disease as a new window of opportunity for therapeutic intervention.

We are currently in "the tools and targets" phase. Work from the study of metastasis suppressors demonstrated that at both the cellular and molecular levels metastasis formation is a dynamic process. Therefore, applying the mechanisms governing the growth of the tumor at the primary site to the metastastic process denies its evolutive and active aspects. De facto, metastasis suppressors now have established roles in specific steps of the metastatic cascade such as invasion, adhesion and intravasation (Fig. 2). They exert these different functions during metastasis by affecting downstream effectors of multiple signaling pathways [4]. Moreover, metastasis suppressors present more complex functions than initially thought when they were first discovered. For instance, they present tumor-specific effects (e.g., they will inhibit metastases formation in some type of cancers and not others) and the nature of the signaling pathway activated/inhibited by a specific metastasis suppressor is also dependent on the tumor cell type and the context.

Investigators are now employing a "reverse genetics" approach to discover the phenotypic alterations caused by modulating metastasis suppressor levels and/or activity. This strategy is allowing the identification of tumor host interactions that are required for efficient colonization, the idea being that disrupting these can be targeted to suppress metastases formation. We will first discuss studies addressing invasion and migration, key functions for both early and late in the metastatic process. We will then describe findings involved in lodging and subsequent colonization of the secondary site. The survival of cells within the bloods stream or lymphatics is not addressed, as these steps have thus far been refractory to mechanistic study. Rather than attempt to present a comprehensive overview, we have focused on a few metastasis suppressors that have yielded insight into mechanisms controlling these processes that are required for metastasis formation.

2. Metastasis suppressors in local invasion and intravasation

2.1. Nm23

Nm23 (non-metastatic clone #23) belongs to the nucleoside diphosphate kinases (NDP Kinases) family and was the first identified metastasis suppressor gene in murine melanoma cell lines [2]. Since then, the determination of the exact mechanism through which Nm23 inhibits the metastastic cascade has been proven to be a challenge. Nm23 has a variety of potential in vivo functions that have been reviewed extensively [5]. For the purpose of this review we will focus on its distinct effects on cell motility. In various types of cancers e.g. melanoma [6], glioma [7], breast [6], prostate [8], liver [6,9] and colon cancers [10], Nm23 showed a strong inhibitory function in tumor migration. This migration suppressive role of Nm23 is not mediated by its NDP kinase activity and appears to specifically affect directional cell invasion [6,8]. Interestingly, Nm23-mediated suppression of migration is not correlated with altered expression of the major proteases involved in cell motility such as MMP-2, MMP-9, cathepsin-D, and urokinase plasminogen activator [11,12]. These results suggest that Nm23 impairs cell migration and invasion by regulating intermediate factors involved in cell motility. Our understanding of this process is evolving through the identification of Nm23-interacting partners such as the myosin light chain [9] and the rpS3 ribosomal subunit [13]. One of the most productive lines of inquiry has been the link between Nm23 and the lysophosphatidic acid (LPA) receptor. Specifically, in Nm23-expressing breast cancer cells, transfection of LPA receptor EDG2 rescued the motile and invasive phenotype [14,15]. Expression of EDG2 by Nm23-breast cancer cells increases lung colonization upon spontaneous metastasis assay in vivo [15]. A more recent study suggested that Nm23 blocks the guanine exchange factors (GEF) activity of Dbl-1, inducing an inhibition of Cdc42 activity that ultimately leads to the suppression of tumor cell migration [16]. Although the precise mechanism(s) through which Nm23 suppresses motility remains to be clarified, there has been tremendous progress in our understanding of its function in metastasis and translation of these findings into clinical disease.