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Review

Epigenetic drivers of tumourigenesis and cancer metastasis

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ABSTRACT

Since the completion of the first human genome sequence and the advent of next generation sequencing technologies, remarkable progress has been made in understanding the genetic basis of cancer. These studies have mainly defined genetic changes as either causal, providing a selective advantage to the cancer cell (a driver mutation) or consequential with no selective advantage (not directly causal, a passenger mutation). A vast unresolved question is how a primary cancer cell becomes metastatic and what are the molecular events that underpin this process. However, extensive sequencing efforts indicate that mutation may not be a causal factor for primary to metastatic transition. On the other hand, epigenetic changes are dynamic in nature and therefore potentially play an important role in determining metastatic phenotypes and this area of research is just starting to be appreciated. Unlike genetic studies, current limitations in studying epigenetic events in cancer metastasis include a lack of conceptual understanding and an analytical framework for identifying putative driver and passenger epigenetic changes. In this review, we discuss the key concepts involved in understanding the role of epigenetic alterations in the metastatic cascade. We particularly focus on driver epigenetic events, and we describe analytical approaches and biological frameworks for distinguishing between "epi-driver" and "epi-passenger" events in metastasis. Finally, we suggest potential directions for future research in this important area of cancer research.

1. Introduction

Cancer is a collection of heterogeneous diseases that differ in molecular and phenotypic characteristics. It is one of the leading causes of death worldwide [1] and the number of new cases is increasing. Importantly, 90% of cancer-related deaths occur due to metastasis [2], a hallmark ability of tumour cells to disseminate to distant organs in the body [3]. Therefore, understanding the metastatic process holds a key to preventing metastasis and designing new therapies for advanced cancers. For a long time, cancer was perceived as a disease of the genome, predominantly resulting from mutations in key genes. However, a voluminous amount of literature in the last two decades has demonstrated that epigenetic changes are associated with almost every step of tumour development and progression [4]. It is now established that genetic and epigenetic changes share an intricate relationship in the tumour evolution process [4].

Our current knowledge of epigenetic aberrations in cancer cells is based primarily on the initial events associated with tumourigenesis [5]. In contrast, relatively little is known about the key epigenetic events that lead to tumour progression and metastasis. Although primary tumour cells harbour an array of cancer-specific genetic mutations, less than 0.01% of the primary cancer cells that enter circulation are able to metastasise [3]. This suggests that a subset of primary tumour cells needs to acquire additional changes to enable them to successfully metastasise. An alternative model is that a series of stochastic cellular events allow the primary tumour cells to disseminate out to and survive in distant organs, eventually leading to the formation of metastatic lesions [6]. Extensive sequencing efforts indicate that gene mutations alone may not explain metastasis [7]. It is now becoming increasingly evident that epigenetic changes play a key role in providing additional properties to the primary cancer cell and these events have a major contribution to the metastatic process [2,8,9]. Metastasis is a multi-step process and cancer cells exhibit dynamic phenotypic transitions during these events [10]. Unlike genetic events, epigenetic changes are also dynamic and therefore it is intriguing to begin to appreciate that primary tumour cells almost certainly use epigenetic machinery to achieve metastatic properties.

In cancer biology, the idea of a "driver" is used to define crucial genetic mutations that initiate tumourigenesis *i.e.*, genetic mutations that directly or indirectly provide a selective growth advantage to the cancer cell are referred to as "driver mutations" [7]. Two major classes of genes that harbour driver mutations and confer a growth advantage

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to cancer cells are oncogenes and tumour suppressor genes (TSGs) [7]. Activating mutations in oncogenes and/or mutations that silence TSGs allow cancer cells to escape the normal checkpoints of cell division and promote tumour growth [7]. However, a recent global consortium felt that it was necessary to extend this definition to comprehensively describe the more complex biology of cancer cells [11]. A cancer driver event was defined as the alterations (cell-autonomous or non-cell-autonomous) that contribute at any stage of tumour evolution and progression [11]. This definition includes and recognises the contribution of non-genetic (*e.g.*, epigenetic) changes as potential driver events in cancer initiation and progression.

One bottleneck in studying epigenetic events potentially involved in cancer metastasis is the lack of an analytical framework for identifying driver epigenetic changes. For genetic studies, the framework for discriminating a driver change (which provides a causal selective advantage to the cancer cell) from a passenger change (which is a consequential change) is relatively well established [7]. As epigenetic marks are dynamic and tissue specific, the number of epigenetic alterations in a cancer genome is potentially much higher than genetic alterations [4,9]. Therefore, it is important to establish a framework for identifying epigenetic drivers of metastasis. In this review, we discuss the current understanding of the epigenetic basis of cancer metastasis. We particularly focus on driver epigenetic events and we describe analytical approaches and the potential tenets of a framework for distinguishing “epi-driver” and “epi-passenger” events in metastasis. We have compared and contrasted relative contributions and interrelationships of genetic and epigenetic changes where applicable. We mainly focus on DNA methylation events as methylation is a stable and somatically heritable mark and the majority of current understanding of epigenetic events in metastasis is based on methylation analysis. However, where relevant, we discuss gene expression patterns and chromatin changes.

2. Is there such a thing as an epigenetic driver of cancer metastasis?

2.1. Epigenetic silencing of TSGs and activation of oncogenes as potential driver events

Methylation of a CpG dinucleotide is a stable and mitotically heritable epigenetic mark. Aberrant global DNA methylation patterns in human cancers were first reported in 1983 [12], just a year after the discovery of the *RAS* oncogene in human cancer cell lines [13,14]. The discovery of oncogene mutations opened up a new field and subsequently intensive research efforts have been directed towards understanding the function of oncogenic mutations in driving tumorigenesis, while much less research on epigenetic events in cancer occurred during this time. The first gene-focussed evidence in favour of the role of DNA methylation in cancer came from a study of retinoblastoma patients. This study identified focal hypermethylation in a promoter associated CpG island (CGI) in the retinoblastoma suppressor gene (*RB* gene) [15]. *RB* is a key TSG that suppresses excessive cellular growth by inhibiting cell cycle progression. Following this discovery, CGI hypermethylation and methylation-mediated inactivation of the Von Hippel-Lindau (*VHL*) gene was reported in clear-cell renal carcinomas [16]. Since then numerous other studies over the last two decades have shown that hypermethylation of promoter CGIs is a major mechanism of silencing TSGs in cancer cells and this phenomenon has now been documented in almost every cancer type [5]. In normal somatic cells, almost 70% of CpG sites are methylated, but gene promoter associated CGIs remain relatively unmethylated. However, in cancer, despite the observation that for a number of genes the specific CGIs that are proximal to gene promoters undergo a gain in DNA methylation associated with gene silencing, there are a number of studies that suggest that it is only in the minority of genes that CGI methylation correlates with repression of the corresponding gene expression [5]. Nevertheless, currently, promoter DNA methylation mediated gene silencing is by far the most recognised epigenetic event in cancer.

For several decades, cancer has been perceived as a disease of our genes, predominantly caused by mutations. Although epigenetic alterations are an almost universal feature of all cancer types [5], whether these changes are causal or merely a consequence of primary genetic alterations in cancer cells has been debated until recently. The conceptualization of an epigenetic driver is therefore relatively recent and several independent lines of evidence provide a strong basis for this concept [2,9]. Consistent findings of focal hypermethylation in key genes (especially in TSGs) leads to the hypothesis that if epigenetic changes are a driving process in cancer onset and progression, then driver genes can be silenced by an epigenetic mechanism [4,17]. However, epigenetic silencing of passenger genes may also occur due to aberrant regulation of epigenetic machinery, and these changes are not necessary for cancer to occur [18]. To test this hypothesis, the global DNA methylation in colon cancer cell lines was experimentally depleted by disrupting two major methylation machinery enzymes (*DNMT3B* and *DNMT1*). Although methylation levels substantially decreased globally, several genes responsible for cancer cell survival retained their hypermethylated state [19], suggesting that methylation-dependent silencing of key genes was required for the cancer cells to survive. Another key TSG, p16 *(*encoded by the *CDKN2A* gene in humans), regulates exit from G1 phase to S phase during cell division. Silencing of p16 is one of the most well documented and early epigenetic events and is reported in all common human cancers [20]. *cis*-Acting DNA sequences were engineered to specifically hypermethylate the p16 promoter in mice, resulting in transcriptional suppression of the gene in somatic tissues during aging. The mice with a hypermethylated p16 promoter had a higher incidence of developing spontaneous cancer [21]. In addition, mice that carried inactivating germline mutations in one allele of p16 and epigenetic alterations in the other allele showed early onset of tumours and reduced tumour-free survival, demonstrating that epimutations are able to act as driver events in tumour initiation and progression. Although this is a good example of an epimutation that is able to act as a driver event, additional examples from *in vivo* experiments are needed to establish this paradigm beyond doubt.

DNA methylation is reversible and therefore, in contrast to hypermethylation and inactivation of TSGs, removal of methylation marks can lead to activation of potential oncogenes. However, the main focus of many studies for many years has been the epigenetic control of tumour suppressor genes, whereas hypomethylation directed activation of oncogenes has been relatively less studied. The first hypomethylation and concordant high-level expression was reported for the *BCL2* gene in lymphocytic lymphoma [22]. Following that, unmasking of methylation marks and activation of the proto-oncogene *HOX11* was reported in lymphoid tumours [23]. Subsequently, loss of methylation marks and activation of other proto-oncogenes such as *RRAS* in gastric cancers [24]. The same phenomenon has also been reported for *MAGE* family genes and *GPR17* in head and neck and lung cancers [25]. An important observation has been CpG demethylation in the hypoxia response element of *HIF-1α*, which is normally silenced by methylation. Demethylation of the promoter enabled HIF-1α to bind to its own promoter leading to an autotransactivation of HIF-1α expression [26]. Over-expression of HIF-1α has critical implications for cancer growth as it is involved in energy metabolism, angiogenesis, survival, and the tumour invasion process [27]. Although demethylation had been reported in different cancers, how methylation marks were removed in an active and replication independent manner was not known until 2009 [28,29]. The discovery that the TET family of enzymes can oxidise 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) provided a mechanism to explain the active demethylation process. In this mechanism, the 5hmC intermediates are actively converted to unmodified cytosines through successive rounds of oxidation by TET and then base excision repair by thymine DNA glycosylase [30]. Recent work has indicated that hypoxia induced loss of TET activity can result in