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Mini-review

MAGE-A antigens as targets in tumour therapy

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ABSTRACT

MAGE-A proteins constitute a sub-family of Cancer-Testis Antigens which are expressed mainly, but not exclusively, in germ cells. They are also expressed in various human cancers where they are associated with, and may drive, malignancy. MAGE-A proteins are highly immunogenic and are considered as potential targets for cancer vaccines and/or immuno-therapy. Moreover, recent advances in our understanding of their molecular pathology have revealed interactions that offer potential as therapeutic targets. Here we review recent progress in this area and consider how these interactions might be exploited, especially for the treatment of malignant cancers for which available treatments are inadequate.

1. Melanoma-associated antigens (MAGE)

Melanoma-associated antigens (MAGE) were initially discovered as tumour-associated antigens in melanoma patients [1] but it is now clear that these proteins comprise a super-family of more than 60 genes in humans [2,3]. The MAGEs are subdivided into two groups, MAGE-I and MAGE-II, based on the chromosomal locations of the genes and the tissue distribution of their products [2,3]. MAGE-I proteins are members of the broader family of *Cancer-Testis (CT) Antigens*. Physiologically, they are expressed mainly in germ cells and trophoblasts. Additionally, certain MAGE-A proteins may have specific functions in particular somatic tissues (such as MAGE-A11 in prostate and MAGE-A3 in thyroid [4,5]). Moreover, the recent demonstration that individual MAGE family members have specific partners among the RING-finger E3 ligase family is also consistent with the idea that they may have specific functions in somatic tissues albeit at possibly low levels [6]. Specific MAGE-A proteins are also expressed briefly during early embryonic development in certain somatic cells such as the spinal cord and brain stem [7]. From a cancer perspective, however, their expression is reactivated in developing cancer cells and is often associated with the onset of malignancy. MAGE-I comprises three multi-gene families, MAGE-A, -B, and -C, each of which is encoded as a separate cluster on the X chromosome. Excluding pseudo-genes, there are eleven genes in the MAGE-A family (termed MAGE-A1 to -A12 [MAGE-A7 is a pseudogene]), nine in MAGE-B and four in MAGE-C. MAGE-II genes are not normally associated with cancer, have a broader chromosomal distribution, and are expressed at various levels in many normal tissues [2]. They are thought to function in diverse biological processes including neuronal differentiation and apoptosis. Given the advances of the past few years in understanding the molecular biology of MAGE-A proteins and their potential role as drivers of metastasis, this review will focus, for the most part, on members of this sub-family.

2. MAGE-A proteins and cancer

2.1. MAGE-A expression in cancer

MAGE-A proteins are now known to be highly expressed in a wide range of cancers including breast, ovary, lung, and bladder (for detailed reviews see [3,8,9]). Given that MAGE proteins are highly conserved, studies have been limited by the inherent difficulty in developing specific antibodies for individual family members and many groups have tried to circumvent this issue by measuring the mRNA levels of specific MAGEs; (see Suppl. Fig. 1 and Suppl. Table I respectively for a lineup of the MAGE-A protein sequences and a list of commonly used anti-MAGE-A antibodies). Additionally, the large

Abbreviations: AR, androgen receptor; ARF, alternative reading frame; CHK1, checkpoint kinase 1; CT, cancer/testis; EBV, epstein-barr virus; EGF, epidermal growth factor; HDAC, histone deacetylase; hNSE1, human non-SMC element 1 homologue (*S. cerevisiae*); KAP1, (also known as TRIM28); KZNF, KRAB (Krueppel-associated box)-type zinc finger; MAGE, melanoma antigen; MDM2, murine double-minute, clone 2; MHC, major histocompatibility complex; MHD, MAGE homology domain; NRAGE, neurotrophin receptor-interacting MAGE homologue; PHD2, hypoxia-inducible prolyl hydroxylase-2; PML, pro-myelocytic leukaemia; RBCC, RING, B-box, coiled- coil domain; RING, really interesting new gene; RNAi, RNA interference; SKIP, SKI-interacting protein; SMC5/6, structural maintenance of chromosomes genes 5 and 6; SV40, simian virus 40; TRIM28, (also known as KAP1).

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number of MAGE genes (and indeed non-MAGE CT antigens) that can be expressed in cancers means that it is extremely difficult to assess expression of all the relevant genes in all cancers. However, in spite of these hurdles, a number of important conclusions can be drawn from the wealth of publications reporting their expression. For instance, cancer cells generally tend to co-express combinations of two or more MAGE-A (or other CT) antigens in a manner that is unlikely to be random; for example, combinations frequently include MAGE-A3 or MAGE-C2 suggesting a possible selection for these proteins [3]. Additionally, MAGE-A expression is observed mainly in cancers that have acquired malignant phenotypes, e.g. invasiveness or metastasis, an observation that is true for a range of cancer types [3]. MAGE detection also correlates with poor prognosis in cancer patients, underpinning the idea that MAGE proteins may contribute actively towards malignancy.

2.2. Effects of MAGE-A expression in cell- and animal models for cancer

The notion that MAGE-A expression is associated with, and may actually help drive, malignancy, is supported, in principle, by a number of laboratory-based studies. For example, MAGE-A3 expression stimulates cell cycle progression, migration rate and invasion of thyroid cells *in vitro*, characteristics normally associated with aggressive behaviour in tumour cells [4]. Moreover, expression of MAGE-A3 in an orthotopic xenograft model for thyroid cancer leads to increases in tumour size and in the number and size of metastatic foci in the lung [4]. Similarly, elimination of expression of Mage-B in murine melanoma cells can suppress melanoma growth in xenografts [10]. Clearly, therefore, the mouse model studies that are available to date support an active role for MAGE proteins in promoting malignancy in at least two different cancer types. MAGE-A expression can promote additional malignancy-associated characteristics in cultured cells. For example, several MAGE-A proteins can confer resistance to clinically relevant chemotherapeutic drugs by modulating the p53 pathway [11–14]. Additionally, cell lines that express MAGE-A proteins show resistance to TNF-induced cytotoxicity [15].

2.3. Mechanisms of MAGE-A expression in cancer cells

The mechanism(s) by which MAGE-A expression occurs in tumour cells is understood, at least in part. Expression of MAGE-A and other MAGE-I genes in somatic tissues is repressed by DNA hypermethylation of CpG dinucleotides in promoters which acts to prevent access of transcription factors such as Ets and SP1 [16,17]. In tumour cells, however, genome-wide epigenetic reprogramming can result in promoter hypo-methylation leading to the aberrant expression of one or more of these genes. Consistent with this model, MAGE-A expression can be induced by demethylating agents such as 5-aza-2′-deoxycytidine in non-expressing cells of various origins [16,17]. It is also apparent that other chromatin remodelling events which occur during tumour development, such as histone acetylation and methylation, may also contribute to cancer-related changes in MAGE-A levels and, at least in some circumstances, can be under control of hormones such as FGF, estrogen or leutenising hormone [5,18,19]. Interestingly, MAGE expression may also be regulated directly by microRNAs such as miR-34a [14]. It is therefore possible that combinations of changes in regulatory events contribute to the variability of expression of the different MAGE-A genes in cancer cells.

Cancers also show significant variations in nuclear versus cytoplasmic distribution of MAGE-A [8] suggesting that either different MAGE-A family members exhibit differential localisation or that the mechanisms controlling localisation are tumour cell-specific. Localisation could be a factor in defining the outcome of MAGE expression, depending, of course, on the localisation of relevant MAGE target- or interacting proteins.

Addressing these issues will be challenging but is likely to be important because, if there are subtle, but significant, variations in function and/or expression between different MAGE proteins, the development of therapeutic strategies may well depend upon targeting the right MAGE protein(s) in the right tumour type(s). Alternatively, if common features can be characterised fully, these may provide avenues for universally targeting MAGE proteins during cancer development.

3. Biological function of MAGE-A proteins

Our current understanding of the biological function(s) of the MAGE-I proteins is limited and, to date, studies have focused largely on their role(s) in the molecular pathology of tumour development (see below). In addition to cancer, however, there is a need to understand how they contribute to germ cell biology and other biological events in which they are involved. One of the major challenges in assessing this role is just the sheer number of genes that need to be examined and their high degree of similarity to each other. Tackling this issue even by state-of-the-art mouse knockout technology is significantly challenging. Thus, whether MAGEs share a broad biological function(s) and operate redundantly or whether they have specific or selective roles, albeit subtle ones, remains unclear for the present. Based on the established expression of many MAGE-A proteins in the spermatogonia, roles in proliferation, migration, de-differentiation and stem cell function have been suggested [3,20]. However, to date, the precise nature of such functions, and whether they are shared by different family members, remains obscure. There is also growing evidence for selective roles for specific MAGE-A proteins in other tissues. For example, MAGE-A11, which is unusual in that its expression is extended to other tissues of the male and female reproductive tracts, may function in androgen signalling and embryo implantation respectively [5]. Similarly, MAGE-A3 is present in normal thyroid tissue where it is a target, and potentially a downstream component, of FGFR signalling that influences cell growth [19,21]. In contrast to the MAGE I family, there is a better understanding of the biological involvement of members of the MAGE II sub-families. Interestingly, although not thought to be directly involved in cancer, several of the MAGE II proteins have been implicated in cancer-related events. For example, hNSE3/MAGE-G1 is part of the SMC5–6 complex which is essential for proliferation and regulates chromatin dynamics in response to DNA damage [22,23]. MAGE-G1 may function, at least in part, by stimulating the ubiquitin ligase activity of the NSE1 component of this complex [6]. MAGE-D1 (NRAGE), which interacts with several proteins including the p75 neurotrophin receptor and inhibitor of apoptosis proteins (IAPs), blocks cell cycle progression and enhances apoptosis in neural progenitors [24,25]. MAGE-D4 (Magphinin) is thought to regulate cell proliferation during gametogenesis [26] while Necdin has an important neuron-specific role in the development/maintenance of specific cell types where is acts as a growth suppressor [27].

Unravelling the biological functions of the MAGE proteins, while of significant interest in its own right, could provide enormous insight into their ability to promote aggressive cancer development. Moreover, the identification of specific partners for individual MAGE proteins (see below) may help lead the way towards improving our knowledge in these areas.

4. Molecular functions and interactions of the MAGE proteins

At the molecular level, MAGE-A proteins have been implicated in two broad activities, each of which may impinge on both the biolog-