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Review

Aberrant *PAX3* and *PAX7* expression. A link to the metastatic potential of embryonal rhabdomyosarcoma and cutaneous malignant melanoma?

J. Blake and M.R. Ziman

School of Biomedical and Sports Science, Edith Cowan University, Joondalup, Western Australia, Australia

Summary. Transcription factors encoded by *PAX3* and *PAX7* are amongst the first expressed in the embryo, being principal regulators of neurogenic and myogenic progenitor cell specification and embryonic segmentation. The basis for this review lies in the supposition that genetic programs for cell migration, thought regulated by *PAX3* and *PAX7* during embryonic development, become tools used by the metastatic cell. In highly metastatic neoplasms arising from cells of neurogenic and myogenic lineages such as embryonal rhabdomyosarcoma and cutaneous malignant melanoma, markedly high expression levels of *PAX*3 and *PAX*7 support this supposition.

As *PAX*3 and *PAX*7 are known to play a role in the regulation of migratory events in embryogenesis, it is possible that the metastatic potential of these tumours is directly linked to migratory properties conferred them through *PAX* expression.

Here we provide a novel perspective by correlating metastasis with expression of *PAX*3, *PAX*7 and ephrin/Eph receptors as well as NCAMs, cell surface markers normally involved in migration and adhesion during development, and propose a role for *PAX* genes in the increased metastatic potential of these tumours.

Key words: Developmental genes, Metastasis, *Pax* genes, NCAM and ephrins

Introduction: Scope of the review

Ultimately, it is the metastatic tumour cell that proves most dangerous to the cancer sufferer. The horrifying implications that metastatic spread has on the prognosis for a cancer victim, compels study into the phenotype of the metastatic tumour cell; presumably, a greater understanding of this phenotype will lead to improved treatment regimes.

It is known that approximately 1 in 10,000 metastatic cells survive and only those disseminated cells that express the correct combination of gene products will *successfully* metastasise. Furthermore, metastatic cells endure a process of events similar to those that occur in development of the embryo, specifically, delamination, migration through tissues and adhesion at secondary sites. Both embryonic and metastatic cells undergo these processes while in a relatively undifferentiated state.

The supposition that genetic programs regulating migration during embryonic development become tools of survival for the metastatic cell forms the basis for this review. In particular, cell, surface molecules known to provide migratory and adhesive properties to cells within the embryo are examined for their ability to increase the metastatic potential of cells within neoplasms.

Genes that play a role in early embryonic patterning, such as the *Hox* and *Pax* developmental genes, are key factors in cell differentiation and migration. Here we specifically review the role of *PAX*3 and *PAX*7 in regulation of cell surface molecules involved in the migratory process.

Moreover, the relationship between *PAX* genes and cell surface molecules such as Eph receptors, ephrins and NCAM will be examined in two neoplasms having a highly metastatic phenotype, embryonal rhabdomyosarcoma (ERMS) and cutaneous malignant melanoma (CMM).

Observations that *PAX*3 and *PAX*7 have been found over-expressed in ERMS, while *PAX*3 is over-expressed in CMM, have led to the suggestion that increased *PAX* expression leads to up-regulation of key cell surface molecules, thereby conferring migratory properties to neoplasms which influence their metastatic potential. We predict that such information will provide valuable

Offprint requests to: Dr. Mel R. Ziman, School of Biomedical and Sports Science, Edith Cowan University, 100 Joondalup Drive, Joondalup, Western Australia, 6027, Australia. Fax: 61-8-94005717. email: m.ziman@ecu.edu.au

Abbreviations: *PAX*, gene encoding the human transcription factor PAX; *Pax*, gene encoding the murine transcription factor Pax; Eph receptor, cognate tyrosine kinase for ephrin ligand; NCAM, neural cell adhesion molecule.

insights for the induction of successful treatments of metastatic tumours.

Embryonal rhabdomyosarcoma

Characteristics

Rhabdomyosarcoma is a soft tissue sarcoma arising from rhabdomyoblasts (a primitive skeletal muscle cell with eosinophilic cytoplasm). There are two subtypes of rhabdomyosarcoma – alveolar (ARMS) and embryonal (ERMS); the embryonal form is the most common, presenting in 70-80% of cases (Bridge et al., 2002). Within the tumour, cells are committed to a myogenic lineage but arrested prior to terminal differentiation (Astolfi et al., 2001). The presence of rhabdomyoblasts suggests that the tumours originate from the same embryonal mesenchymal stem cells as striated skeletal muscle cells.

Cytogenetics

Although cytogenetic studies reveal no specific chromosomal aberration for ERMS, a hyperdiploid karyotype is a persistent feature (Polito et al., 1999; Middel et al., 2000; Bridge et al., 2002); one study revealed cells containing 100 chromosomes (Olegard et al., 1992). Hyperdiploid states reported thus far show gains of chromosomes 2, 7, 8, 11, 12, 13 and 20. Within the hyperdiploid karyotype chromosomal losses can occur; chromosome losses reported to date include chromosomes 1, 6, 10, 14, 15 and 16 (Polito et al., 1999).

Prognosis

ERMS is the most common soft-tissue sarcoma of childhood affecting children between the ages of 2-19 years. The prognosis for ERMS depends largely on the site affected. ERMS that arise in the orbit/eyelid, parameninges, bladder or prostate, have a better prognosis (3-year survival rate of 72%, Baker et al., 2000) than those arising in the CNS, for which the prognosis is extremely poor with survival beyond 24 months being rare (Celli et al., 1998).

The most frequent sites in which secondaries arise are the regional lymph nodes, lungs, liver, bone marrow, bones, and brain (Altman and Schwartz, 1983) with 83% of metastases becoming manifest within one year of diagnosis.

The high expression levels of *PAX* genes within ERMS may be linked to the observed increase in expression of important cell migratory molecules, NCAM and more significantly, polysialylated NCAM (PSA-NCAM) (Phimister et al., 1994; Gluer et al., 1998a,b). The ability of *PAX* genes to confer a high metastatic potential to ERMS cells via up-regulation of cell surface molecules will be discussed in detail later in this review.

Cutaneous malignant melanoma

Characteristics

Cutaneous malignant melanoma (CMM) is a highly metastatic tumour arising from cutaneous intraepidermal melanocytes. In normal development, melanocytes originate from neural crest cells, a unique population of embryonic stem cells that arise along the lateral aspects of the neural folds. During embryonic neurulation, crest cells detach and migrate to target locations where they undergo terminal differentiation.

Cells that give rise to CMM usually progress from a pre-existing melanocytic nevus (congregations of benign melanocytes located in the epidermis and/or dermis) (Zhao et al., 2002). Although there are genetic influences associated with an increased risk of developing melanoma, the increased incidence of melanoma in recent generations has been directly linked to the independent risk factor of excessive sun exposure. It is believed that an initial high dose of ultraviolet radiation causes substantial damage to the melanocyte, resulting in mutations that disrupt the DNA without subsequent apoptosis (Gilcrest et al., 1999).

Cytogenetics

Although the main cause of CMM is thought due to ultraviolet radiation, 10% of CMM cases are familial (Fountain et al., 1990). An inherited syndrome thought to increase susceptibility to CMM, is Dysplastic Nevus Syndrome (DNS), an autosomal dominant inherited disposition with patients possessing 10-100 large nevi or moles (Greene et al., 1985; Bale et al., 1986).

To date, the two principal loci known to be associated with familial inheritance of CMM are at 1p (*CMM*1) and 9p (*CMM*2). Through multipoint linkage analysis, Bale et al. (1989) mapped *CMM*1 to 1p36. The locus denoted *CMM*2, at 9p21, contains several genes considered as candidates for involvement in CMM (Puig et al., 1995). While *CMM*1 is involved in the early stages of CMM, tumour suppressor genes at 9p are thought involved in the pre-disposition and progression of CMM (Puig et al., 1995). There is also thought to be a third locus for *CMM* on chromosome 6 (Dracopoli et al., 1987).

Prognosis

Early stage melanomas are highly curable by complete surgical excision resulting in a greater than 95% eight-year survival rate. However, if lymph node metastases are present, the 5-year survival of patients is between 30% and 50% (Zettersten et al., 2002).

Progression of CMM is thought linked to changes in expression of cell surface molecules involved in the migration of neural crest progenitor cells. Several studies of CMM have shown dramatic changes in expression patterns of NCAM isoforms (Mooy et al., 1995; Reed et

al., 1999) as well as Eph receptors and ephrins (Easty et al., 1997, 1999; Bittner et al., 2000; Easty and Bennett, 2000; McArdle et al., 2001).

In this paper we explore the possible link between the cellular origins of ERMS and CMM and their high metastatic potential. These tumours arise from mesenchymal and neural crest cell lineages, respectively; during development both express *PAX*3 and *PAX*7 and are characterised as migratory. Since recent evidence suggests that *PAX* genes regulate expression of cell surface molecules important in migration, we discuss the possibility that metastatic potential is associated with continued, increased or altered *PAX* gene expression in tumours.

PAX3 and *PAX7* genes

Structure

The *PAX* family of genes derive their name from the paired box which consists of a 384 bp DNA sequence encoding a highly conserved DNA binding domain (Burri et al., 1989; Krauss et al., 1991). The nine members of the gene family are further classified into groups (I-IV) according to possession of a full or partial homeodomain, a DNA binding domain also found in homeobox genes (Bopp et al., 1986). Furthermore, *PAX* genes may contain a conserved octapeptide region (Burri et al., 1989) thought to participate in methylation of the genes (Ziman and Kay, 1998) (Fig. 1).

Both Group III genes, *PAX*3 (2q35, Ishikiriyama, 1993) and *PAX7* (1p35-1p36.2, Schäfer and Mattei, 1993) are very similar in structure; both genes encode the paired box in exons 2-4, the homeobox in exons 5-6 and the transactivation domain in exons 6-8 (Jostes et al., 1990; Vorobyov et al., 1997). The paired box is the most similar in sequence, bar a few differences in the region encoding the carboxyl end of the paired domain (Jostes et al., 1990).

The functions of PAX3 and PAX7 in embryogenesis

All *PAX* genes encode a family of transcription factors that function in the highly specific, spatio-temporal regionalisation of the embryo (for review see Gruss and Walther, 1992; Chalepakis et al., 1993; Mansouri et al., 1994). Pax3 and Pax7 are amongst the earliest transcription factors present during embryogenesis (Jostes et al., 1990; Goulding et al., 1991; Mansouri et al., 1996). Within the neural tube, where they play a role in dorso-ventral patterning, both genes are expressed in cells that specify dorsal neurons (Jostes et al., 1990; Goulding et al., 1991). Their expression patterns in the developing brain correlate with subdivision and specification of brain regions (Stoykova and Gruss, 1994). In particular, expression has been detected laterally in the alar plate of the mesencephalon (midbrain) and in the ventricular zone and mantle layer of the tectum. In the mesencephalon *Pax7* expression is associated specifically with differentiation of the tectum as well as formation of its laminar structure, being expressed in neuronal cells that migrate toward the most dorsal layer, the stratum griseum superficiale (Kawakami et al., 1997).

Pax3 and *Pax7* also function in specification and migration of neural crest derived cells; *Pax3* is involved in the migration and terminal differentiation of melanocytes and Schwann cells (Kioussi et al., 1995) whereas *Pax7* is involved in development of cranio-facial structures (Mansouri et al., 1996).

Finally, *Pax3* and *Pax7* are expressed in myogenic precursor cells and play a role in the migration of these cells from the somatic dermamyotome to the limb buds during myogenesis (Jostes et al., 1990; Kawakami et al., 1997; Tremblay et al., 1998; Henderson et al., 1999; Swartz et al., 2001). Both genes are expressed prior to *MyoD* expression and it is believed that both factors are down-regulated upon terminal differentiation of the cells into skeletal muscle cells (Williams and Ordahl, 1994; Marcelle et al., 1995). The life-long persistent expression of *Pax7* in satellite cells (a population of myogenic stem cells) is believed responsible for maintenance of the satellite cells in an undifferentiated state (Seale et al., 2000).

The importance of *Pax* genes in specification and migration of neural, neural crest and myogenic cell lineages is highlighted by the mutated phenotype. Mutations in *PAX3* result in Waardenburg syndrome (Hoth et al., 1993; Tassabehji et al., 1993), characterised by hearing loss as well as pigmentation abnormalities

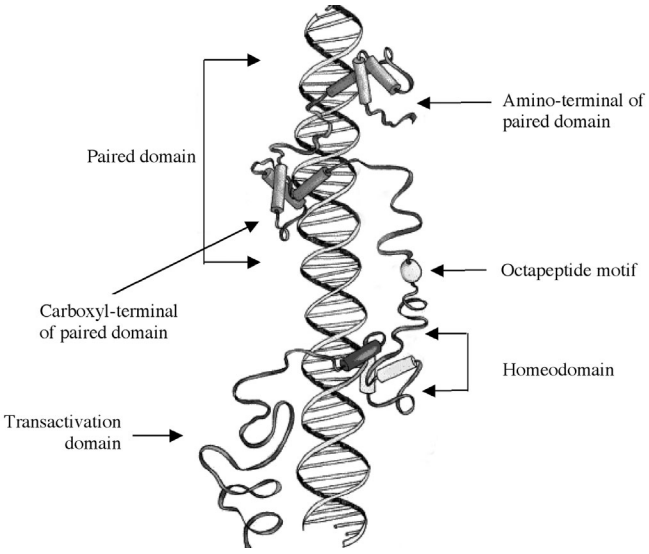

Fig. 1. Pax3 and Pax7 contain a paired DNA domain domain consisting of amino- and carboxy-terminal subdomains, each of which is composed of three helices in a helix-turn-helix motif. The third helix of each subdomain is shown in contact with the DNA. The Pax proteins also contain an octapeptide followed by a homeodomain containing an additional helix-turn-helix DNA binding domain. Finally, the transactivation domain is indicated.

due to the absence of melanocytes.

The *Pax3* mutant, Splotch mouse (the term 'Splotch' refers to coat colour abnormalities resulting from melanocytic defects) exhibits severe defects in the formation and migration of myoblasts (Franz, 1990), neural crest derived Schwann cells, dorsal root and cranial ganglia and melanocytes (Epstein et al., 1991).

Pax7$^{-/-}$ mice may develop to term; however, they are characterised by growth retardation, severe defects to the maxilla and nasal structures (Mansouri et al., 1996) and small musculature due to a complete lack of muscle satellite cells (Seale et al., 2000).

Pax3 and Pax7 transcripts

Functional diversity of *Pax*3 and *Pax*7 genes *in vivo* is thought linked to the ability to produce alternately spliced gene products that alter the structure and consequently the binding activity of the paired and homeodomain regions (Tsukamoto et al., 1994; Underhill and Gros, 1997; Seo et al., 1998; Ziman and Kay, 1998). The resultant isoforms encoded by alternate *Pax* transcripts are postulated to bind to a variety of target gene sequences, by alternate use of the paired domain, the homeodomain or both, thus activating a variety of downstream pathways (Underhill and Gros, 1997; Vogan and Gros, 1997) (Fig. 2).

Originally both *PAX3* and *PAX7* genes were thought to contain only eight exons. It is now known that the coding region of *PAX3* consists of ten exons (Barber et al., 1999) and the coding region of *PAX7* consists of nine or more exons (Barr et al., 1999).

Recent studies show that *PAX3*/*Pax3* may be alternately spliced at exons 4, 5, 8, and 9 to produce six different transcripts (Barber et al., 1999) including transcripts that lack an entire homeodomain (Tsukamoto et al., 1994) (Fig. 3). These alternate *PAX3* transcripts encode proteins with different binding and transactivation activities having an important effect on the functional role of the gene (Underhill and Gros, 1997)

Four distinct *Pax7* transcripts that contain structural differences in the paired box have been described previously (Ziman et al., 1997; Ziman and Kay, 1998; Kay and Ziman, 1999). These transcripts are expressed in a cell/tissue specific manner, and are thought to play a role in differentiation of cells along neurogenic and myogenic lineages (Ziman and Kay, 1998; Ziman et al., 2001). Novel transcripts of *PAX7* that contain an additional ninth exon have been found expressed in human tumour tissue (Barr et al., 1999) and in vertebrate species such as the zebrafish (Seo et al., 1998). Whether transcripts containing the ninth exon are expressed in normal human tissue is at present unknown and currently under investigation. Changes at the 3' end of the gene are likely to affect the activity of the transactivation

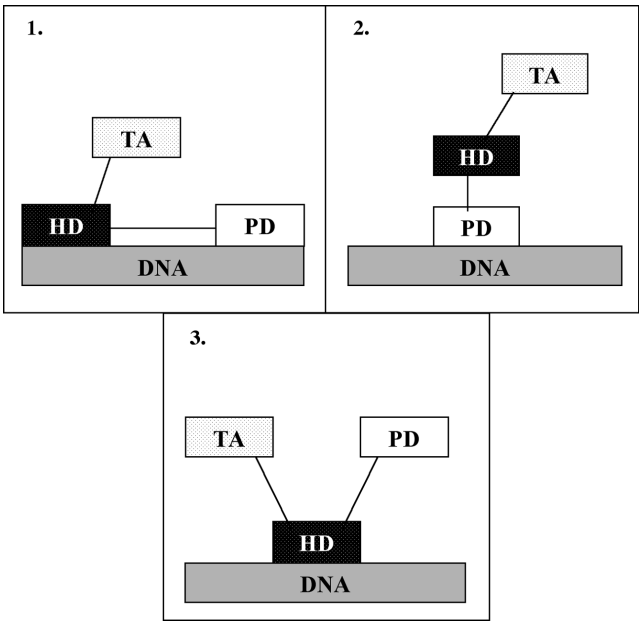

Fig. 2. A schematic diagram indicating possible modes of binding by alternate Pax3 or Pax7 isoforms to target DNA. Alternate Pax isoforms are produced by alternate splicing at intron-exon boundaries during transcription of the *Pax*3 or *Pax*7 genes. 1) Pax protein binding to target DNA using both the paired and homeodomains. 2) Pax protein binding to target DNA using either the paired domain or 3) the homeodomain.

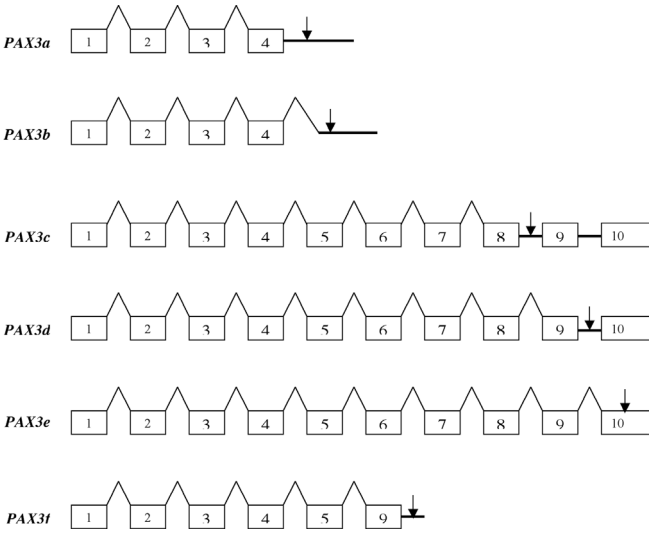

Fig. 3. Alternate transcripts of *PAX3* produced by alternate splicing at intron-exon boundaries. The predicted locations of translation termination are indicated by an arrow for each transcript. *PAX3a* and *PAX3b*: These transcripts lack the entire homeodomain and c-terminus (Tsukamoto et al., 1994). *PAX3c*: Transcript containing 8 exons and a stop codon 5 bp into intron 8 (Goulding et al., 1991). *PAX3d*: Transcript contains exon 9 spliced directly onto the 3' end of exon 8 (Barber et al., 1999). *PAX3e*: Transcript containing 10 exons (Barber et al., 1999). *Pax3f*: Transcript found in murine cDNA contains exon 9 directly spliced to the 3' end of exon 5 (Barber et al., 1999).

domain within the encoded proteins.

PAX genes and cancer

Mutations in *PAX* genes are associated with development of a variety of cancers such as astrocytoma, medullablastoma, lymphoplasmacytoid lymphoma, Wilm's tumour, melanoma, sarcoma, rhabdomyosarcoma and thyroid cancer (Dressler and Douglas, 1992; Kozmik et al., 1995; Stuart et al., 1995; Iida et al., 1996; Schulte et al., 1997; Kelly et al., 1998; Barr et al., 1999; Vachtenheim and Novotna, 1999; Kroll et al., 2000; Scholl et al., 2001).

Established associations of *PAX* genes with cancer include chromosomal translocations that in turn encode oncogenic fusion proteins. The *PAX3-FKHR* t(2;13) and *PAX7-FKHR* t(1;13) translocations are responsible for alveolar rhabdomyosarcoma (Bennicelli et al., 1996), the *PAX5* t(9;14) translocation plays a role in lymphoplasmacytoid lymphoma (Iida et al., 1996) and the PAX8-PPARd fusion oncoprotein in thyroid carcinoma (Kroll et al., 2000).

Although mutations in *PAX* genes are associated with the many forms of cancer described above, this is not the case in ERMS and CMM, where the causative mutations are at gene loci other than those of the *PAX* gene family. Of note, however are the recent findings by Barr et al. (1999) showing increased and aberrant expression profiles of *PAX3* and *PAX7* transcripts in these tumours. These recent investigations were based on the reasoning that expression of *PAX* genes in myogenic and neural crest progenitors might be repeated in tumour cells arising from the same lineage. Using RNAse protection assays to quantify *PAX3* and *PAX7* expression in both cell lines and tumour tissue from ERMS and CMM, levels of *PAX3* and *PAX7* in ERMS, and *PAX3* in CMM were found to be "notably high".

In these cancers, the predominant *PAX3* and *PAX7* transcripts contain a previously uncharacterised ninth exon (Fig. 4). Additional *PAX3* and *PAX7* transcripts resulting from alternative splicing at exon 8 were also observed (Barr et al., 1999). These findings raise the possibility that the metastatic phenotype of ERMS and CMM may be specifically related to the increased, aberrant expression of specific *PAX* transcripts.

How would these transcripts play a role in tumour metastasis? Aberrant *PAX* expression has been demonstrated to affect activation of downstream target genes associated with cell proliferation and adhesion. In CMM, altered *PAX3* expression is consistently associated with loss of expression of the *Mitf* gene and subsequent proliferation leading to melanoma formation (Galibert et al., 1999; Vachtenheim and Novotna, 1999; Kamaraju et al., 2002). *Mitf* is essential for melanocyte differentiation and plays a role in the regulation of genes required for melanogenesis (Bentley et al., 1994; Yasumoto et al., 1997).

Moreover, aberrant *PAX3* expression has been correlated with up-regulation of *ST8SiaII/STX*, a gene involved in the post-translational sialylation of NCAM. Increased NCAM sialylation results in decreased cell adhesion and increased cell migratory properties. (Mayanil et al., 2000).

The ability of *PAX* genes to affect cell surface molecules involved in regulation of cell motility and adhesion has important implications for metastasis. We next review *PAX* gene regulation of cell surface molecules, particularly those involved in delamination, migration and adhesion during embryogenesis, as they provide interesting links to metastatic capabilities.

Fig. 4. Diagram indicating alternative *PAX3* and *PAX7* transcripts found expressed in ERMS and CMM relative to normal tissue. The transcribed 3' regions of the genes are shown as closed boxes and attached horizontal lines, respectively. The paired box is designated PB, the homeodomain is designated HD and exons are labelled with dashed vertical lines. **A)** *PAX3* and **C)** *PAX7* cDNA transcripts found in normal tissue containing exons 1-8. **B)** Predominant *PAX3* and **D)** predominant *PAX7* transcripts found expressed in ERMS (*PAX*3 and *PAX*7) and CMM (*PAX3*). The dark area indicates the position at which the alternately spliced exon 9 is attached to an alternative 3' splice site in exon 8 (adapted from Barr et al., 1999)

Cell surface molecules associated with migration

Eph receptors and ephrins

Important biochemical cellular surface molecules that function in the migration of embryonal cells are ephrin ligands and their cognate family of Eph receptors. Eph/ephrin complexes regulate morphogenesis of the embryo by affecting the adhesive and/or repulsive properties of the migrating cell.

The Eph receptor family of tyrosine kinases consists of at least fourteen receptors and nine ligands (Eph Nomenclature Committee, 1997). Class A receptors bind to GPI-anchored ligands whereas class B receptors bind to transmembrane ligands; EphA4 is the only receptor with an affinity for both classes of A and B ligands (Gale et al., 1996).

Mechanisms of Eph receptor/Ephrin induced migration

Induction of either a repulsive or attractive signal in

the cell cytoplasm is achieved by activation of protein tyrosine kinases that catalyse tyrosine phosphorylation of substrate proteins (Wybenga-Groot et al., 2001). Eph receptor mediated repulsion functions through an intricate system of initial binding, cleavage by a metalloprotease, shedding of the ephrin ectodomain and subsequent cell retraction or detachment and movement (Krull, 1998; Hattori et al., 2000). Alternatively, resistance to proteolysis may transduce adhesive signals to the cytoplasm. Class A ephrins containing shorter membrane regions (Lai et al., 1999) become resistant to metalloproteases due to inaccessibility of the protease to the proximal region of the ligand (Schlondorff and Blobel, 1999).

Recently, Honda and Mochizuki (2002) proposed a bilateral threshold control mechanism to explain Eph/ephrin mediated cellular repulsion or adhesion; cells adhere to each other if their surfaces bear a critical density of ligand which is reciprocal to the density of the receptor, whereas, cells are repelled if the density of ligands is unequal to the critical threshold.

Eph receptors and ephrins in embryogenesis

There is much evidence to support a role for Eph/ephrin complexes in mediation of cell movement during embryonic pattering, axonal pathfinding, vascular development and angiogenesis (Iwamasa et al., 1999; Marin et al., 2001; for review see Cheng et al., 2002; Coulthard et al., 2002).

Of particular interest to our study are the situations in the developing embryo where Eph/ephrin mediated repulsion results in de-adhesion, collapse of cell processes and cell detachment. It has been observed that Eph/ephrin binding results in cleavage and shedding of the ephrin ectodomain allowing cell detachment to occur (Krull, 1998; Hattori et al., 2000). This process of collapse and retraction has been observed in developing axons (Oakley et al., 1993) and individual cells of the migrating neural crest (Krull, 1998). Collapse and retraction of cells due to Eph/ephrin signalling may be comparable to the detachment of the metastatic cell from a primary tumour.

It is possible that the adhesive role of Eph/ephrin signalling also has implications in metastasis. During embryogenesis, Eph/ephrins induced cell adhesion is important for digit formation (Staedler et al., 2001), closure of the neural tube (Holmberg et al., 2001) as well as angiogenesis (Pandey et al., 1995). In angiogenesis, Eph/ephrin signalling activates cell adhesion to extracellular matrices via integrin-dependent mechanisms (Huyn-Do et al., 1999; Miao et al., 2000).

The endothelium of blood vessels contains a physical barrier that metastatic cells must penetrate in order to invade the underlying tissue. Thus, the presence of Eph receptors and ephrins, located on both endothelial cells of vascular vessels and disseminated tumour cells, may be an important part of the mechanism by which

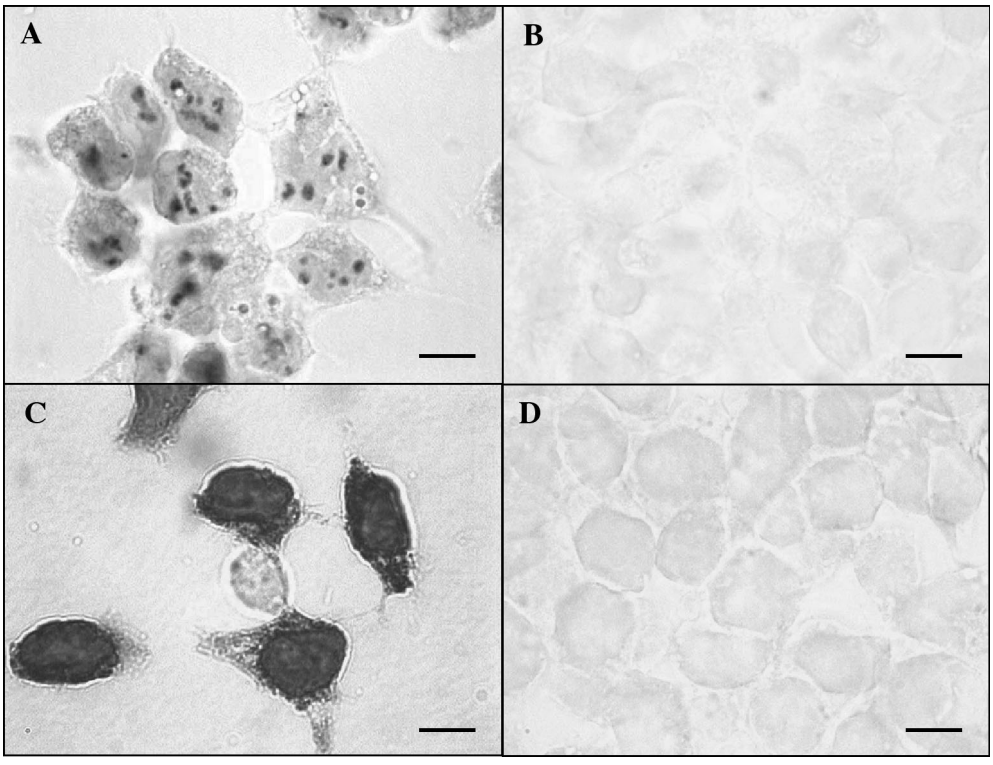

Fig. 5. Immunohistochemistry to detect downstream target genes of Pax 7. P19 cells, transfected with a *PAX7*-pHM6 vector construct or with pHM6 vector alone, were fixed in 4% paraformaldehyde and gene expression was assessed using Pax7 and ephrin-A2 antibodies. **A.** Positive *PAX7* expression was observed in the nuclei of P19 cells transfected with *PAX7* but was not observed in **B,** undifferentiated cells transfected with pHM6 vector. **C.** *Ephrin-A2* expression was observed in P19 cells transfected with *PAX7* but no *ephrin* expression was present in **D** pHM6 vector transfected cells. Scale bar: 20 μm. Similar results were obtained in three experiments performed in three or more separate clones. (From Thomas et al., 2001).

tumour cells pass through the endothelial junction, thus increasing the metastatic cell's ability to extravasate.

Pax genes and regulation of Eph receptors and ephrins

Studies in our laboratory as well as others, demonstrate a close association between *Pax* gene and *Eph*/*ephrin* expression. *Pax7* and *ephrin-A2* co-localise in spatiotemporal patterns to neurones of the superficial layers of the superior colliculus (Kawakami et al., 1997; Marin et al., 2001) and a recent study performed in our laboratory demonstrated that cells transfected with *PAX*7 *in vitro* consistently up-regulated *ephrin-A2* (Thomas et al., 2001) (Fig. 5).

In a similar manner, the EphA4 receptor is thought to play a role in migration of *Pax7* expressing muscle precursor cells to the limb bud during embryonic development (Swartz et al., 2001), while *Pax6* is thought to regulate *EphB2* expression in retinal ganglion cell neurons (Ziman et al., 2003). Thus the ability of *PAX* genes to up-regulate the *Eph* receptors and *ephrins* may play a role in developmental migration of neural crest and myogenic cells along clearly defined paths.

Eph receptors, ephrins and cancer

Considering the importance of ephrins and Eph receptors in normal cell signalling, adhesion and migration it is highly probable that they are key determinants in the metastatic process. In fact, numerous reports detail the over-expression of *Eph* receptors, such as *EphA2*, *EphA3* and *EphB2* in various tumour types including melanomas (Easty and Bennett, 2000; Lawrenson et al., 2002), sarcomas (Chiari et al., 2000), small cell lung cancer (Tang et al., 1999) and breast cancer (Nikolova et al., 1998). Not surprisingly, higher expression levels of *Eph* receptors are found to correlate with more malignant and metastatic tumours (Easty et al., 1995; Zelinski et al., 2001).

EphA2 is consistently over-expressed in 90% of metastatic melanoma cell lines (Easty et al., 1999) whereas *EphA2* expression is not detected in normal melanocytes (Easty and Bennett, 2000). Coincidently, Scholl et al. (2001) reported that *PAX*3 expression is similarly confined to malignant metastases with no *PAX3* expression in the surrounding normal tissue or benign nevi.

These results imply that up-regulation of *PAX* genes in metastatic cells may in turn activate expression of *Eph* receptors and *ephrins* thereby increasing the ability of the cell to migrate in a manner similar to that of embryonic cells. Further research to establish a link between *PAX* genes and *Eph*/*ephrins* in metastatic tumours is currently being performed in our laboratory.

Neural cell adhesion molecules

Another important cell surface molecule that is crucial for cell migration of embryonal cells is the neural cell adhesion molecule (NCAM). Of particular significance to the present study is its previously identified role as a downstream target of several *Pax* genes (Edelman and Jones, 1995; Wang et al., 1996; Holst et al., 1997).

Definition, structure and function

The neural cell adhesion molecule (NCAM) is classified as a member of the immunoglobulin superfamily (Edelman and Crossin, 1991); it functions in morphogenic patterning through the mediation of homophilic or heterophilic binding between cells. NCAMs span the cellular membrane, have a short cytoplasmic tail and contain five Ig-like repeats that are expressed on the extracellular domain of the protein (Springer, 1990).

NCAM exists as several isoforms- 120-kDA, 140-kDA and 180-kDA (Cunningham et al., 1987; Roesler et al., 1997; Perl et al., 1999). NCAM-120 contains a unique exon; when included in the transcripts, this exon encodes a plasma membrane GPI anchor (Roesler et al., 1997). NCAM-140 and NCAM-180 contain trans-membrane components encoded by an additional exon. The 180-kDA isoform occurs predominantly on differentiated cells interacting with spectrin to bring about cellular adhesion to the basal lamina or to adjacent cells (Roesler et al, 1997).

Polysialylation of NCAM

A post-translational modification of NCAM that directly affects its adhesive properties is polysialylation. Polysialic acid is a large negatively charged homopolymer and its linkage to NCAM negates the kinetics of homophilic dimerisation promoting heterophilic binding of NCAM to extracellular matrix proteoglycans (Rutihauser et al., 1988; Storms and Rutihauser, 1998).

The polysialylated form of NCAM (PSA-NCAM) is expressed in a large variety of tissues in the embryo and is associated with increased cellular mobility. As the brain develops, PSA-NCAM augments cellular motility, assisting neuron outgrowth. Conversely, PSA-NCAM is not present within the adult brain other than in areas requiring synaptic regrowth such as the hippocampus and olfactory bulb (Tanaka et al., 2000).

PAX proteins and regulation of *NCAM*

The *NCAM* gene contains a binding site for the paired domain of Pax (PBS) (Holst et al., 1997) and two homeodomain binding sites (HBS) (Wang et al., 1996). Pax proteins are thought to regulate *NCAM* expression via the PBS and HBS sequences in its promoter. The PBS promotor region of *NCAM* consists of two half sites that are identical to the consensus binding sequences of various Pax proteins (Wang et al., 1996). Moreover,

Pax3 has been shown to regulate *NCAM* expression by binding at PBS half sites (Chelapakis et al., 1994).

NCAM and Cancer

The importance of NCAM in conferring oncogenic properties to a neoplasm is highlighted by studies that show tumours lacking the 180-kDa isoform demonstrate clinically aggressive behaviour that is associated with metastatic disease or patient death within 18 months of presentation. The presence of the 180-kDA isoform of NCAM in tumours is associated with non-aggressive clinical behaviour (Roesler et al., 1997).

Furthermore, although PSA-NCAM expression is no longer present in most adult tissues, re-expression of the polysialylated form of NCAM has been demonstrated in malignant tumours such as small-cell lung cancer, neuroblastoma, alveolar rhabdomyosarcoma and medullablastoma (Soler et al., 1993; Kojima et al., 1997; Gluer et al., 1998a,b; Mayanil et al., 2000; Tanaka et al., 2000).

Of particular interest to this study is the fact that mutation of *PAX* genes results in increased expression of polysialylated NCAM (Fukuda et al., 2000). In a study of medullablastoma, increased presence of polysialylated NCAM was associated with an increased expression of the *STX* gene which was further attributed to an over-expression of *PAX*3 (Mayanil et al., 2001). These results indicate that aberrant expression of *PAX* genes may affect NCAM and PSA-NCAM expression and in turn affect metastatic potential.

Conclusion

ERMS and CMM have the propensity to be more dangerous than several other forms of cancer due to their ability to invade surrounding tissues and metastasise early in their development. Generally, about 50% of patients have metastatic spread at the time of clinical detection (DeVita et al., 1975).

Clinical research and animal studies have shown that metastasis is an event that reflects the properties of both the host tissues and the metastatic cell itself (for review, see Fidler, 1978). The ability of a cancer cell to take advantage of genetic programs set in place specifically for normal cellular functions such as cell detachment, migration and adhesion, could give the metastatic cell a tremendous selective advantage.

Future work in the study of the cancer cell phenotype would benefit by assessing expression of genes important for embryonal patterning and migration. Developmental control genes such as the *PAX* family of genes may hold keys to the understanding of oncogenic events such as (and not limited to) metastasis. As we continue to uncover the cellular events that take place during the transformation of the undifferentiated stem cell to the terminally differentiated adult cell, we continue to discover key players that may propagate metastatic cell behaviour.

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