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A Review of the Association between Osteosarcoma Metastasis and Protein Translation

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Summary

The malignant transformation of mesenchymal cells within the bone leads to the development of osteosarcoma (OS), but the genetic underpinnings of these events are not understood. From a clinical perspective, primary tumour management can be achieved successfully in most patients. However, the development of metastasis to the lungs represents the most common cause of death in OS patients. A clearer understanding of metastasis biology is required to improve cancer mortality and improve outcomes. Modelling the genetics, biology and therapy of OS can be accomplished through research involving a number of species. Most notable is the naturally occurring form of OS that develops in dogs. Through a cross-species and comparative approach important questions can be asked within specific and suitable models to advance our understanding of this disease and its common metastatic outcome. A comparative perspective on the problem of OS metastasis that utilizes a cross-species approach may offer unique opportunities to assist in this prioritization and generate new hypotheses related to this important clinical problem.

Keywords

dog; metastasis; osteosarcoma; protein translation

Osteosarcoma

Osteosarcoma (OS) is an uncommon tumour of bone, arising from the malignant transformation of mesenchymal cells, which differentiate towards the formation of osteoid and bone (Malawar *et al.*, 2005; Kansara and Thomas, 2007). While OS can arise in any bone, the most common sites of primary tumours are the distal femur, proximal tibia and proximal humerus. Beyond primary tumour growth in the appendicular and less commonly the axial skeleton, a defining feature of OS biology is its high propensity for pulmonary metastasis (90% of metastases are to the lung; Krishnan *et al.*, 2005). These defining features of OS are shared between human and canine OS patients. This cross-species review describes the disease with an emphasis on metastasis biology and our interest in the potential linkage between efficient protein translation and metastasis.

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Conflict of Interest

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Human Osteosarcoma in Paediatric Patients

OS may occur at any age; however, it has a peak incidence in the second decade of life with a second smaller peak of incidence in the elderly population (>50 years of age) (Hayden and Hoang, 2006; Kansara and Thomas, 2007). Although rare, it is the most common paediatric bone malignancy in the USA (Gurney *et al.*, 1999). Approximately 800–1,000 cases of OS are diagnosed in the USA each year and about half of these patients are teenagers (Wang, 2005; Kansara and Thomas, 2007). OS occurs most frequently during the adolescent growth spurt in areas of rapid bone growth, suggesting a relationship between tumour formation and rapid growth and/or growth factors expressed at this time in life (American Cancer Society, <http://www.cancer.org/docroot/home/index.asp>). OS may occur more frequently in males than females (60% versus 40%) and slightly more often in African Americans than Caucasians (Gurney *et al.*, 1999).

Fifty years ago, when surgery was the only available treatment, a diagnosis of OS was often fatal. Patients had only a 15–20% chance of cure (Dahlin and Coventry, 1967; Hayden and Hoang, 2006). Fortunately, advances in chemotherapy, including the introduction of single agent and combination chemotherapy, and advanced orthopaedic surgical techniques in the 1980s and 1990s increased survival rates dramatically. Today, patients with localized disease at presentation have a 65–70% chance of 5-year relapse-free survival (Bielack *et al.*, 2002; Hayden and Hoang, 2006; Kansara and Thomas, 2007; Meyers, 2009). Unfortunately, the remaining patients will relapse (with pulmonary metastasis) within the first 5 years, attesting to the fact that these patients have undetectable metastatic disease at diagnosis. Indeed, up to 20–25% of newly diagnosed patients have detectable metastases at diagnosis (Hattinger *et al.*, 2010). Patients presenting with metastases have a poor prognosis, with long-term survival rates of 10–30% (Meyers, 2009). Other prognostic indicators for early relapse include the size and location of the primary tumour, the response to preoperative (neoadjuvant) chemotherapy and surgical success (Bielack *et al.*, 2002). Current chemotherapy treatment plans are based on neoadjuvant chemotherapy followed by surgical removal of the tumour and post-operative (adjuvant) chemotherapy. The use of neoadjuvant chemotherapy has the advantage of down-staging the size of the primary tumour and as such providing a more successful opportunity for primary tumour resection with limb salvage. Pathological analysis of the definitive resection provides an opportunity to evaluate histologically chemotherapy-induced tumour necrosis in the resected specimen.

The Huvos grading system (Huvos *et al.*, 1977) is a non-invasive quantitative estimate of the percentage of tumour cell necrosis (death) observed in the tumour after surgery. Patients showing tumour necrosis in at least 90% of the resected tumour specimen are classified as good responders (Huvos score 3 or 4) and have more favourable prognoses than patients with less necrosis. Patients with low necrosis scores (Huvos score of 1 or 2) are often selected to receive alternative and more aggressive chemotherapy protocols in the adjuvant setting. Patients who achieve a good histological response to preoperative chemotherapy have considerably better survival than those who have a poor response. Five-year survival for those with good response is approximately 75–80%, compared with 45–55% for those with poor response (Whelan *et al.*, 2000; Bielack *et al.*, 2002). Exemplary of this approach, the European and American Osteosarcoma Study (EURAMOS) group, has launched, and now nearly completed, the EURAMOS I study. In this study histological response (Huvos score) following preoperative chemotherapy (<http://www.ctu.mrc.ac.uk/euramos>) is used to assign patients to distinct treatment groups. The current treatment backbone used as first-line therapy in patients includes cisplatin, doxorubicin and methotrexate. The EURAMOS objectives are: (1) to examine whether the addition of ifosfamide and etoposide (IE) to postoperative chemotherapy improves event-free survival for patients with a poor histological response to preoperative chemotherapy and (2) to examine whether the addition

of interferon (IFN)- α as maintenance therapy after post-operative chemotherapy improves event-free survival for patients with a good histological response to pre-operative chemotherapy.

Despite sophistication of trial designs and multimodality treatments for OS, improvements in long-term survival in the last 20 years have been modest at best. The primary cause of death for patients continues to be the development of metastasis. In order for patient outcomes to improve, we must improve our understanding of the biology of metastasis. Given the complexity of metastasis, there remains a need to develop or to identify reliable animal models of OS. An ideal model would include development of spontaneous primary bone tumour and pulmonary metastases within an immunocompetent host. This type of model would provide the best opportunity to identify key regulatory pathways involved in the development and metastatic progression of OS, as well as the ability to investigate activities of novel antimetastatic therapeutics.

Comparative Studies in Animals

Cross-species comparative opportunities to understand OS biology and therapy are strong. Current animal models of OS include rats, mice and dogs. Each model provides unique strengths. Rat OS models play an important role in evaluating new surgical and molecular methods of treatment for appendicular OS (Blouin *et al.*, 2005). Mouse models of OS are used commonly to study tumour biology and develop new approaches for treating OS. Transplantable mouse OS models include syngeneic (genetically identical or closely related, so as to allow successful tissue transplant), xenograft (derived or obtained from an organism of a different species, as a tissue graft; human explants), heterotopic (pertaining to the injection of cancer cells/tissue to a place it is not normally found; e.g. non-osseous tissues) and orthotopic (pertaining to the transplantation of cancer cells/tissue into its normal anatomical position; e.g. bone microenvironment). Mouse syngeneic models of OS are especially valuable for studying primary tumour and metastatic progression. These models utilize an immunocompetent mouse host that provides the advantage of mimicking the complex interactions between tumour cells and the relevant host microenvironment (Fan, 2010). While excellent for evaluating primary tumour and metastatic progression, syngeneic models lack the species-specific information that is critical to OS pathogenesis in people. As such, xenograft models of OS are commonly used for studying human OS biology and new anticancer treatments. In xenograft models, human OS cells are injected into immunodeficient mouse hosts; therefore, a weakness of the model is that it fails to mimic accurately important aspects of the host microenvironment–tumour cell interactions that participate in primary tumour development and metastatic OS progression (Fan, 2010). In mouse models of OS, tumour cells can be transplanted orthotopically into the intermedullary cavity or paraosseous structures of either the proximal tibia or distal femur or heterotopically to the subcutis or musculature. Orthotopic transplantation of OS cells more closely replicates critical host stromal cell–tumour cell interactions that likely participate in OS pathogenesis and biology (Fidler *et al.*, 1990; Fu *et al.*, 1991; Fan, 2010). Currently, syngeneic, orthotopic transplantation mouse models are the most common experimental system used to study OS biology (Fidler *et al.*, 1990; Khanna *et al.*, 2000; Hoffman, 2001). Finally, genetically engineered mouse (GEM) models provide an experimental system to model the genetics of human OS.

The most successful GEM models regulate the genetic modification (i.e. knock-in or knock-out) in a tissue-specific manner through tissue-specific promoters or tissue-specific recombination events. In OS, conditional knockout mouse models represent an experimental system that supports endogenous, spontaneous generation of tumours through knockouts of critical tumour suppressor genes. These models have proven very useful in identifying

regulatory pathways in OS tumourigenesis and metastatic progression as well as providing a valuable platform for developing novel therapeutic strategies (Khanna *et al.*, 2000; Berman *et al.*, 2008; Walkley *et al.*, 2008). Conditional knockout is based on a tissue-specific inactivation of the gene of interest. This can be achieved by means of Cre-lox recombination, a genetic technology that allows site-specific recombination events including deletions (Sauer and Henderson, 1988). While the aetiology of OS is unknown, it is evident that the tumour suppressor genes *p53* and *Rb* (retinoblastoma) play a role, as children with familial mutation syndromes affecting either of these genes have higher incidences of OS (Hansen, 1991). Two GEM models lacking the *p53* and *Rb* genes have been created using Cre-loxP recombination strategies. These models produce F1-generation mice that readily develop OS; however, while *p53* loss is associated with the development of OS, the *Rb* gene mutation alone is not sufficient to induce osteosarcomagenesis. Instead, it must act synergistically with *p53* to induce osteosarcomagenesis (Berman *et al.*, 2008; Walkley *et al.*, 2008). These models replicate many defining features of human OS including gene-transcription signatures; however, the observed biological behaviour of OS generated in the mice does not consistently mimic all aspects of the disease in man (Walkley *et al.*, 2008).

Companion animals naturally develop OS and the disease is almost identical in dogs and people. As such, the dog represents a naturally occurring and spontaneous metastatic animal model of OS (Vail and MacEwen, 2000; Withrow and Khanna, 2009; Fan, 2010; Paoloni *et al.*, 2010). The clinical presentation, biology, treatment, complications and outcomes are similar in both dogs and people, although human patients have better outcomes and the incidence of OS is much higher in dogs (Withrow and Wilkins, 2010). Another noteworthy difference in canine OS is that the median age of onset is in adulthood. In addition, physal plates in dogs (versus children) are closed at the age of onset of OS in the breeds of dogs that are overrepresented for OS development (Fan, 2010; Withrow and Wilkins, 2010). Despite these differences, the biology and genetics of OS are highly conserved between people and dogs (Paoloni *et al.*, 2009).

Importantly, the canine OS model includes spontaneous and natural development of the primary tumour and pulmonary metastases within an immunocompetent host. The increased risk of OS development in specific breeds of dogs has raised the possibility that the dog with OS may be useful in identification of genes linked to cancer formation. The opportunity for studies of OS biology and genetics has been enhanced through the development of the Canine Comparative Oncology and Genomics Consortium (CCOGC). The CCOGC has developed a national canine OS biospecimen repository (the Pfizer Canine Comparative Oncology and Genomics Consortium Biospecimen Repository). This US biospecimen repository seeks to collect cancer, normal tissues and biofluids from over 1,800 dogs with a variety of cancers including approximately 600 dogs with OS. Release of tissues from this repository, based on meritorious scientific proposals, is expected before the end of 2012. The translational opportunity to better develop new cancer treatments for OS patients through studies of dogs with OS, has been widely recognized (Paoloni and Khanna, 2008; Withrow and Khanna, 2009). Clinical trials conducted by single institutions and multicentre studies are conducted commonly in dogs with OS with this intent. These studies are increasingly integrated within existing drug development plans of cooperative groups and pharmaceutical companies interested in OS treatments for human patients (Khanna, 2008).

Metastasis

As suggested above, the single most significant negative prognostic factor in the treatment of cancer is the development of metastatic disease. Treating metastatic OS remains a challenge for oncologists. In part this is the result of the differences in the biological behaviour of metastatic tumours from primary tumours (Krishnan *et al.*, 2005; Kansara and

Thomas, 2007; Bacci *et al.*, 2008; De Nigris *et al.*, 2008; Guo *et al.*, 2008; Posthuma-DeBoer *et al.*, 2011). As such, the therapeutic regimens that target primary tumours may not be successful in the treatment of metastatic disease. The primary research need in the field is to understand the biology of metastasis in OS and to use this understanding to develop therapeutics that target the metastatic phenotype.

Dissemination of cancer may occur through one of three pathways: (1) direct seeding of body cavities or surfaces, (2) lymphatic spread and (3) haematogenous spread (Slauson and Cooper, 2002; Kumar *et al.*, 2005). Haematogenous spread is typical of solid tumours such as OS. The metastatic cascade is an extremely complex multistage process. In order for a metastatic lesion to become clinically detectable, it must successfully complete a sequential series of steps of the metastatic cascade. Within this process, each step is subject to a wide variety of influences; thus, at any point in the sequence the tumour cells may not survive. Failure to complete any step results in failure to colonize a distant site. The metastatic cascade can be divided into two phases: (1) invasion of the extracellular matrix (ECM) and (2) vascular dissemination and arrest in a distant site (Kumar *et al.*, 2005). Prior to invasion of the ECM, clonal expansion, growth, diversification and angiogenesis must occur in order to produce metastatic subclones within the primary tumour (Krishnan *et al.*, 2006). These 'transformed' tumour cells must then separate from the primary tumour and interact with the ECM at several stages in the metastatic cascade. Integrins are adhesion molecules that promote stable interactions between cells and their environment. They bind to ECM proteins and send anchorage-dependent signals that can influence cell survival by suppressing apoptosis (Griffiths *et al.*, 2011). Apoptosis results from a loss of integrin-mediated cell attachment to the ECM.

In order for the neoplastic cells to breach the underlying basement membrane they must first detach themselves from one another and their surroundings. After the cells are detached, it is necessary for them to attach to matrix components (e.g. laminin, collagen and fibronectin) so that the basement membrane can be degraded enzymatically to create passageways for tumour cell migration (Slauson and Cooper, 2002). The basement membrane is degraded by serine, cysteine and matrix metalloproteinases (MMPs). Loss of either cell-cell or cell-matrix interaction activates caspase proteases, the hallmark of apoptosis (Frisch and Francis, 1994). Tumour cells resist detachment-induced apoptosis (anoikis) by establishing contacts with other tumour cells (homotypic interactions) or with host cells such as platelets and inflammatory cells (heterotypic interactions). Both types of interactions generate intracellular signals that prevent anoikis (Grossmann, 2002; Krishnan *et al.*, 2006). Furthermore, there can be overexpression of essential proteins by cancer cells that directly inhibit anoikis. TrkB, a tyrosine kinase receptor (Douma *et al.*, 2004), the integrin pair $\alpha_2\beta_3$ (Ruoslahti and Reed, 1994), adenomatous polyposis coli (APC) gene product (Beavon, 2000), focal adhesion kinase, galectin-3 and transforming growth factor- β (Krishnan *et al.*, 2006) are reportedly involved in resistance to anoikis.

Once tumour cells have invaded the surrounding tissues and migrated through the basement membranes into the bloodstream (lymphatics or peritoneal space), they are susceptible to destruction by innate and adaptive immune defences. In addition, destruction of intravasated tumour cells by haemodynamic forces and shearing is thought to contribute to metastatic inefficiency (Weiss *et al.*, 1992). However, it has been demonstrated recently that some tumour cells in the bloodstream can arrest in capillary beds and extravasate with high efficiency (Luzzi *et al.*, 1998). Neoplastic cells must strategically evade detection and destruction by the immune system and other antitumour effectors at all stages of metastatic progression (Slauson and Cooper, 2002; Hunter, 2004; Kumar *et al.*, 2005; Krishnan *et al.*, 2005, 2006).

Adhesion among tumour cells together with adhesion between tumour cells and platelets allows tumour cells to aggregate in clumps (tumour emboli) within the bloodstream (Kumar *et al.*, 2005). These platelet–platelet aggregates may enhance tumour cell survival and implantation. Arrest and extravasation of tumour emboli at distant sites involves adhesion to the endothelium followed by exit through the basement membrane. Involved in this process are adhesion molecules such as integrins and laminin receptors and previously mentioned proteolytic enzymes (Slauson and Cooper, 2002). At the new site, the tumour cells need to survive, proliferate, develop a vascular supply and avoid and survive apoptotic signals and host immune responses. The site at which the circulating tumour cells leave the capillaries to form secondary deposits is defined, in part, by the anatomical location of the primary tumour, the associated venous drainage and the first capillary bed encountered by metastatic cells (Weiss *et al.*, 1988; Kumar *et al.*, 2005). With venous invasion, neoplastic cells follow the venous flow draining the site of the tumour; hence, the liver and lungs are most frequently involved secondarily in haematogenous dissemination (Kumar *et al.*, 2005). Arterial dissemination may occur when tumour cells pass through the pulmonary capillary bed or when pulmonary metastases give rise to additional tumour emboli. It is important to note, however, that venous drainage and capillary bed proximity alone does not define the sites of metastasis. Currently, it is believed that the specific site for distant metastasis is not simply due to an anatomical location, the primary tumour or proximity to secondary sites, but rather involves interactions between tumour cells and the local microenvironment at the secondary site.

One theory that attempts to explain the selectivity of certain tumour types to metastasize to specific organ sites, for example OS to lung and bone, is the ‘seed and soil hypothesis’ first proposed in 1889 by the surgeon Stephen Paget and then experimentally tested by Fidler in the 1980s (Paget, 1889; Hart and Fidler, 1980). Paget hypothesized that communication between the cancer cell (seed) and the target organ (soil) via molecular interactions resulted in non-random selection of target organs by the cancer cells. Modulation of the tumour microenvironment (soil) by the tumour cell (seed) is a critical determinant of survival of metastatic cells (Paget, 1889). Mendoza and Khanna (2009) recently suggested in their review of cancer metastasis that a critical outcome of the seed–soil interaction is resistance to the stresses tumour cells encounter that would otherwise impede metastatic progression.

The final critical step in the development of metastasis is the generation of a new blood supply (angiogenesis). Proliferation of endothelial cells is a rigidly controlled balance between proangiogenic factors (e.g. vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF] and interleukin [IL]-8) and their antagonists (e.g., thrombospondin-1, maspin and angiostatin) (Slauson and Cooper, 2002; Kumar *et al.*, 2005; Krishnan *et al.*, 2005, 2006). In cancer, this balance is shifted towards proangiogenic factors.

Metastasis, Stress, and Protein Translation

As outlined above, one of the most critical determinants of a tumour cell’s success in metastasis may be its ability to resist the stresses that are associated with progression through the metastatic cascade and survival at secondary sites. Stresses encountered at the secondary site include low oxygen tension (hypoxia), reactive oxygen species, inflammation, nutrient deprivation and changes in pH. These stresses can challenge the growth and progression of a tumour cell and commonly lead to the death of the vast majority of tumour cells that reach a secondary site (Hunter *et al.*, 2008). The ability to detect and overcome these stresses is critical to the metastatic phenotype of cancer. Additionally, it is believed that such stresses may also provide a selective pressure favouring growth of more metastatically ‘fit’ cells (Witz and Levy-Nissenbaum, 2006). The theory that metastatic cells

are programmed or can adapt to the numerous stresses experienced during metastatic progression has been reviewed elsewhere (Mendoza and Khanna, 2009).

Within a dynamic process like metastasis, it is attractive to hypothesize that the ability of a metastatic cancer cell to respond and manage these numerous stresses could be accomplished through an efficient mechanism to deliver critical proteins to specific locations in a cell in a rapid manner. Since it is energetically impossible to express all proteins at all times, a premium would be afforded to cells with such protein translational machinery. More generally, there is a growing body of evidence that suggests links between dysregulation of protein synthesis and malignant progression of cancer. Indeed, the development of rapamycin and its structural analogues as inhibitors of the mTOR pathway (and cap-dependent translation) as anticancer drugs is evidence of the potential clinical value of blocking the protein translation machinery.

From the perspective of this review, activity and target modulation of these inhibitors of translation initiation in OS models has been reported (Wan *et al.*, 2005; Paoloni *et al.*, 2010). Clemens and Bommer (1999) have discussed the many ways in which translational control is relevant to the topic of cancer, including how the efficient expression of important proteins involved in cell growth regulation, proliferation or cell death may be controlled at the translational level by changes in the activity of components of the protein synthesis machinery. Furthermore, mutations that lead to changes in the structure of individual mRNAs may alter the rates at which the proteins encoded by these mRNAs are produced. Finally, disruptions in the regulation of signalling pathways that result in a loss of control of the synthesis of growth-promoting proteins (or impair the synthesis of growth-inhibitory or pro-apoptotic proteins) may alter the balance of production of important cellular components (Clemens and Bommer, 1999).

Translation is the second process of protein biosynthesis, which is part of the overall process of gene expression. In translation, mRNA is decoded to produce a polypeptide based on the genetic code. There are three distinct phases of translation including initiation, elongation and termination. In order for translation to proceed there must first be activation. In activation, the correct amino acid is joined to the correct transfer RNA (tRNA). When the tRNA has an amino acid linked to it, it is termed 'charged'. Once the tRNA has been charged, initiation can begin (Lehninger *et al.*, 1993; Pain, 1996). Initiation involves the small subunit of the ribosome binding to the 5' end of mRNA with the help of other proteins that assist the process (initiation factors [IFs]) (Pain, 1996). Elongation takes place when the next charged tRNA in line binds to the ribosome along with GTP and an elongation factor (EF). Termination of the polypeptide happens when the A site of the ribosome faces a stop codon (Pain, 1996). Of the three phases, initiation is the most important as it is considered to be rate-limiting in the translation process. Cap-dependent translation initiation is the major translation initiation pathway in eukaryotes. All eukaryotic mRNAs present a 5' terminal nuclear modification, the cap structure (Gingras *et al.*, 1999). In translation, the cap structure marks the spot where the small ribosomal subunit (40S) is to be recruited.

Important in this recruitment process is the eukaryotic initiation factor 4F (eIF4F) complex. eIF4F is a 3-subunit complex composed of eIF4E, eIF4A and eIF4G (Gingras *et al.*, 1999; Mamane *et al.*, 2004). eIF4G is a scaffolding protein that brings together eIF4F, as it has two binding sites for eIF4A and one binding site for eIF4E, but more importantly, it bridges the mRNA cap (via eIF4E) and the 40S ribosomal subunit (via eIF3) (Gingras *et al.*, 1999; Mamane *et al.*, 2004). eIF3 is associated with the small ribosomal subunit and plays a role in preventing the large ribosomal subunit from binding prematurely (Fig. 1). eIF4A is an ATP-dependent RNA helicase. Finally, eIF4E is the cap-binding protein, which is a critical node in RNA regulation impacting nearly every stage of cell cycle progression (Mamane *et al.*,

2004) Specifically, eIF4E coordinately promotes the mRNA export of several genes involved in the cell cycle.

A model describing a hierarchy of weakly and strongly translated proteins in cells has emerged (Baserga, 1990; Graff and Zimmer, 2003). These proteins are differentially regulated by translation. The 'strongly translated' proteins are a stable set of 'less regulated' proteins with short, unstructured 5' untranslated regions (UTRs) that are expressed at a basal rate with limited input from external stimuli. In contrast, 'weakly translated' proteins typically have lengthy, G-C rich, highly structured 5'UTRs and are maintained as stable mRNA transcripts. These so-called weakly translated proteins are not translated unless the translational machinery is up-regulated by stimuli such as growth factors or cellular stress (Sonenberg and Dever, 2003; Bjornsti and Houghton, 2004; De Benedetti and Graff, 2004). This hierarchy of proteins provides efficiency in biological systems by eliminating the need for indiscriminate, large-scale translation of all proteins at all times and locations in the cell.

The model of weakly and strongly translated proteins suggests that, under specific signals and requirements, specific proteins can be translated and delivered to specialized locations in the cell (Richter and Sonenberg, 2005). For example, when the cell is exposed to stress or growth factors, there is an increased concentration of unbound (free) eIF4E available to bind with the eIF4F complex creating the specifically enhanced translation initiation that the weakly translated proteins require for efficient translation. The end result is disproportionately enhanced translation of weak proteins (De Benedetti and Graff, 2004). Interestingly, several mRNAs with complexity (lengthy, G-C rich, highly structured) in the 5' UTR (weakly translated proteins) have been associated with cancer (Table 1), including FGF-2 (Nathan *et al.*, 1997a), VEGF (Scott *et al.*, 1998), c-myc (Saito *et al.*, 1983) and cyclin-D1 (Stacey, 2003). Additionally, there are many proto-oncogenes that are regulated at the translational level including c-myc, c-fos and c-jun (De Benedetti and Graff, 2004; Mamane *et al.*, 2004). Such weakly translated and regulated proteins may be ideally suited for rapid expression and delivery to a metastatic cancer cell that is facing a novel stress during metastatic progression.

eIF4E is a 25 kDa mRNA cap-binding phosphoprotein (Rhoads *et al.*, 1993; Sonenburt and Gingras, 1998). eIF4E is an important modulator of cell growth and proliferation. It is the least abundant component of the translation initiation machinery (Rhoads *et al.*, 1993). Within translation initiation, the abundance and activation of eIF4E is considered both rate and process limiting (Rhoads *et al.*, 1993; Sonenburt and Gingras, 1998). Numerous studies have now implicated eIF4E in tumour formation and, potentially, in metastatic progression. Overexpression of eIF4E in the cell lines, NIH3T3, CREF and MM3MG has resulted in cellular transformation and tumourigenesis (De Benedetti and Rhoads, 1990; Lazaris-Karatzas *et al.*, 1990; De Benedetti *et al.*, 1994; Li *et al.*, 2001). Antisense RNA-mediated suppression of eIF4E suppressed proliferation and changed cell morphology in HeLa cells (De Benedetti and Rhoads, 1990) and suppressed soft-agar colonization as well as tumour formation and growth in *ras*-transformed CREF cells (Rinker-Schaeffer *et al.*, 1993). Furthermore, the ability of the *ras*-transformed CREF cells to invade surrounding normal tissues and metastasize was also markedly reduced (Graff *et al.*, 1995). Expression of antisense RNA to eIF4E in human breast, head and neck cancer cell lines suppressed tumour formation and angiogenesis (Nathan *et al.*, 1997a, b; DeFatta *et al.*, 2000). Finally, functional blockage of eIF4E by expressing 4EBP1 can cause reversion of the transformed and tumourigenic phenotype (Rousseau *et al.*, 1996). Overexpression of eIF4E has been documented in human carcinomas of the breast (Kerekatte *et al.*, 1995; Scott *et al.*, 1998; De Benedetti and Graff, 2004), head and neck (Nathan *et al.*, 1997b; Franklin *et al.*, 1999), bladder (Crew *et al.*, 2000), cervix (Matthews-Greer *et al.*, 2005), lung (Rosenwald *et al.*, 2001; Seki *et al.*, 2002), prostate (Graff *et al.*, 2009) and colon and rectum (Rosenwald *et al.*,

1999; Berkel *et al.*, 2001), as well as in non-Hodgkin's lymphomas (Wang *et al.*, 1999) when compared with normal tissues and benign lesions.

Collectively, these data suggest that eIF4E may play a key role in both tumour formation and metastatic progression by specifically enhancing the translation of a subset of key genes (weakly translated proteins) necessary for overriding normal growth constraints (c-myc, cyclin-D1), inducing angiogenesis (VEGF, FGF-2) and facilitating tumour invasion and metastasis (MMP-9, heparanase) (Zimmer *et al.*, 2000; Jiang and Muschel, 2002; Yang *et al.*, 2003). eIF4E enables cells to coordinate efficiently the translation of these needed transcripts during metastatic progression, thus increasing success in the demanding process of metastasis. While there has been a wealth of evidence in both experimental cancer models and in human cancer tissues implicating eIF4E in tumour development and progression, the majority of this work has been conducted in epithelial tumours. Expression and activity of eIF4E in mesenchymal tumours, particularly OS, requires further investigation. We have recently reported on the consistent expression of eIF4E in the majority of human OS primary tumours and metastases (Osborne *et al.*, 2011). Ongoing studies have demonstrated the modulation of the metastatic phenotype in OS model systems following interference of eIF4E expression by shRNA targeting. Based on the multiplicity of stresses faced by metastatic cells, the targeting of a common mechanism by which cells can manage these stresses may be a useful novel therapy. Novel therapies that target the translational machinery or novel approaches to the use of approved pharmaceuticals that interfere with protein translation (i.e. rapamycin) should be considered within a metastasis-specific drug development path for OS. Comparative studies of such novel therapies in a number of model systems may assist in the development of such therapies.

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Fig. 1.

Translational machinery. Under physiological conditions, eIF4E is bound to its inhibitory binding protein 4EBP1. Upon phosphorylation (P) of 4EBP1, eIF4E is liberated from 4EBP1 and able to bind with capped mRNA (7mG) and eIF4G-eIF3-eIF4A subunits, forming the eIF4F complex. eIF4E is the rate-limiting component of the eIF4F complex. eIF4F is a necessary component of the 48S initiation complex. PABP, poly(A) binding protein.

Table 1

Cap-dependent metastasis-associated mRNAs

Function	Metastasis-related gene
Cell proliferation	c-Myc
	CDK2
	Cyclin-D1
	ODC
Angiogenesis	VEGF
	FGF-2
	PDGF
Anti-apoptotic	Mcl-1
	Bcl-2
	Bcl-xL
	Survivin
Invasion	MMP-9
	Heparanase

CDK2, cyclin-dependent kinase 2; ODC, ornithine decarboxylase; PDGF, platelet-derived growth factor; Mcl-1, induced myeloid leukemia cell differentiation protein; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-lymphoma × isoform long.