Epidermal growth factor receptor tyrosine kinase inhibitors and bone metastases: different mechanisms of action for a novel therapeutic application?

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Abstract

The paper by Angelucci *et al.* published in the current issue of *Endocrine-Related Cancer* suggests a potential, novel application of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) in the treatment of bone metastases. Interestingly, activity of anti-EGFR agents on the pathogenesis and progression of bone metastases has been described in previous reports, and a number of different mechanisms seem to be involved in this phenomenon. Anti-EGFR agents have a direct activity on tumour cells in which they produce growth inhibition, apoptosis, and reduced invasive capacity through the inhibition of molecules associated with tissue invasion such as urokinase-type plasminogen activator (uPA) and matrix metalloproteinase (MMP)-9. In addition, these compounds have an anti-angiogenic activity, either direct by affecting the proliferation and survival of endothelial cells, or indirect by blocking the production of vascular endothelial growth factor (VEGF) in bone marrow stromal cells and in tumour cells. Finally, EGFR-TKIs can inhibit recruitment of osteoclasts in bone lesions, by affecting the ability of bone marrow stromal cells to induce osteoclast differentiation and activation. Taken together, these findings strongly support prospective clinical trials of anti-EGFR agents in cancer patients with bone metastases in order to define their role in the management of bone disease.

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Epidermal growth factor receptor (EGFR) and its ligands are expressed in all cell types with the exception of cells of haematological origin (Salomon *et al.* 1995, Normanno *et al.* 2005a). More importantly, expression of these proteins has been found in the majority of human carcinomas. Pre-clinical studies have also demonstrated that autocrine and/or paracrine loops involving EGFR and EGF-like peptides regulate the growth, survival and ability to form metastases of tumour cells. Following this observation, a number of drugs directed against EGFR have been developed, and are currently under clinical investigation or have been approved for treatment of selected tumour types. Among these, tyrosine kinase inhibitors (TKIs) of EGFR have been shown to efficiently block *in vitro* and *in vivo* the activation of the target receptor, and

to significantly inhibit tumour growth in experimental models (Normanno *et al.* 2003). However, clinical studies have generally shown poor activity of these drugs when used as monotherapy in heavily pre-treated cancer patients. The low efficacy of these drugs is likely to be due to the lack of appropriate criteria for selection of patients, although this is an active area of research (Chan *et al.* 2006).

The paper by Angelucci *et al.* published in the current issue of *Endocrine-Related Cancer* suggests a potential, novel application of EGFR-TKIs in the treatment of bone metastases. In this study, Angelucci and co-workers demonstrate that treatment with gefitinib significantly reduces the ability of a highly metastatic clone of PC3 prostate cancer cells (PCb2 cells) to form bone metastases. Different mechanisms

Figure 1 Potential mechanisms of action of EGFR-TKIs on the pathogenesis of bone metastases. Treatment with EGFR-TKIs produces in tumour cells growth inhibition, apoptosis, and reduced invasive capacity through the inhibition of molecules associated with tissue invasion, such as uPA and MMP-9, and with angiogenesis, such as VEGF. EGFR-TKIs have also direct anti-angiogenic activity by affecting the proliferation and survival of endothelial cells. Finally, EGFR-TKIs affect the ability of bone marrow stromal cells to induce osteoclast differentiation and activation, and to sustain neo-angiogenesis.

seem to be involved in this phenomenon. The urokinase-type plasminogen activator (uPA)/uPA receptor (uPAR) system and matrix metalloproteinases (MMPs) have been shown to be involved in the invasive ability of tumour cells and in the formation of bone metastases (Guise & Mundy 1998, Nemeth *et al.* 2002). Treatment with gefitinib significantly reduced the expression and activation of uPA and MMP-9 in prostate cancer cells, and this was associated with a significant decrease in the invasive ability of PCb2 cells. Furthermore, PCb2 cells were found to express higher levels of uPAR as compared with parental PC3 cells, and treatment with uPA increased significantly the levels of activation of the EGFR in PCb2 cells. These findings suggest that the cross-talk between the uPA/uPAR system and the EGFR might sustain the ability of these highly metastatic cells to form bone metastases, and that gefitinib is able to significantly block this mechanism.

The effects of gefitinib on the formation of bone metastases might also be related to its ability to interfere with osteoclast differentiation and activation. The main mechanism responsible for bone destruction in cancer patients is tumour-mediated stimulation of osteoclastic bone resorption (Roodman 2001) (Fig. 1). However, most osteotropic factors produced by tumour cells act indirectly by binding to accessory cells of the bone marrow microenvironment (Roodman

2001). This heterogeneous cell compartment consists of endothelial cells, as well as mesenchymal stem cells (MSCs), which maintain a level of self renewal and give rise to different specialized connective tissue cells including 'reticular cells' and osteoblasts (Clark & Keating 1995, Deans & Moseley 2000). MSCs, marrow stromal cells and osteoblasts support osteoclast differentiation within the bone (Takahashi *et al.* 1988, Udagawa *et al.* 1989, Mbalaviele *et al.* 1999). Two main factors that are produced by MSCs and their progeny are involved in this phenomenon: macrophage colony stimulating factor (M-CSF), which induces proliferation and differentiation of pre-osteoclast cells, and receptor activator of NF- κ B ligand (RANKL) that is involved in fusion and activation of these cells (Boyle *et al.* 2003). A recent paper published by one of us in *Endocrine-Related Cancer* demonstrated that human MSCs express EGFR and its ligands transforming growth factor alpha (TGF α) and amphiregulin (Normanno *et al.* 2005b). More importantly, this paper has shown for the first time that treatment of human MSCs with gefitinib produced a significant reduction in the synthesis of RANKL and M-CSF, and affected their ability to induce differentiation of osteoclast precursors. Angelucci *et al.* (2006) who found that treatment with gefitinib significantly reduced the ability of conditioned medium from prostate cancer cells to

induce expression of RANKL in osteoblasts, have confirmed this observation. It is now well established that EGFR and several of its ligands are expressed by prostate cancer cell lines and human primary prostatic carcinomas (Leverton & Gullick 2000).

The observations described above raise a number of questions. The first is whether the effects on bone metastases are specific for gefitinib, or are shared by other anti-EGFR agents. In this regard, the ability of different anti-EGFR agents to affect tumour cell invasion and metastatization has long been described. For example, treatment of human squamous cell carcinomas of head and neck cells with the anti-EGFR blocking antibody C225 (cetuximab) significantly reduced their ability to invade surrounding tissues, including bone (Huang *et al.* 2002). Interestingly, this inhibition was associated with down-regulation of MMP-9 expression, a phenomenon also observed by Angelucci *et al.* in prostate cancer cells. More recently, EGFR-TKI PKI-166 as a single agent, or in combination with paclitaxel, was found to inhibit growth in the bone of nude mice in both renal cell carcinomas and androgen-independent prostate cancer (Kim *et al.* 2003, Weber *et al.* 2003). Treatment with this drug produced a significant reduction in the levels of EGFR phosphorylation in tumour cells and in endothelial cells within the tumour mass. This observation suggests that the ability of anti-EGFR agents to block tumour growth in bone is related to their effects on both tumour cells and tumour-associated neo-angiogenesis. In agreement with this hypothesis, AEE788, a dual TKI of EGFR and vascular endothelial growth factor receptor (VEGFR), alone or in combination with taxol induced a high level of apoptosis in both tumour-associated endothelial cells and tumour cells, leading to the inhibition of thyroid tumour growth in bone and to preservation of bone structure (Younes *et al.* 2005). In this respect, gefitinib has also been shown to inhibit tumour angiogenesis through direct effects on microvascular endothelial cells that express EGFR and through reduced production of proangiogenic factors by tumour cells (Hirata *et al.* 2002). Furthermore, preliminary findings from the laboratory of one of us suggest that treatment of human MSCs with gefitinib produces a significant reduction in their ability to secrete VEGF and to induce the formation of novel vessels (N Normanno, unpublished observations). Interestingly, it has been demonstrated previously that osteoclasts express VEGFR-1, and that VEGF can induce osteoclast differentiation and activation (Niida *et al.* 1999).

An additional preclinical question to address is whether these preclinical findings might translate into

a clinical benefit for patients. Recent literature is unfortunately littered with pre-clinical hypotheses that have not been confirmed when translated into clinical trials. However, some clinical data are already available that might reveal an activity of EGFR-TKIs on bone metastases. For example, Albain *et al.* (2002) enrolled 12 patients with bone metastases and bone pain in a Phase II study of gefitinib in breast cancer. Surprisingly, 5/12 patients had significant relief of bone pain, leading to the complete withdrawal of all scheduled narcotics in several cases. Due to the impressive effects on bone pain palliation, two patients were maintained on gefitinib despite objective progression of the disease. A significant improvement in bone pain has also been reported in a patient enrolled in a different trial of gefitinib in metastatic breast cancer (von Minckwitz *et al.* 2003). Finally, activity of gefitinib on the progression of bone metastases has been recently described in non-small cell carcinoma patients (D Garfield and G Zampa, unpublished observations).

To summarize these findings, the activity of anti-EGFR agents on bone metastases appears to be related to a number of different mechanisms (Fig. 1): (1) a direct activity on tumour cells in which anti-EGFR agents produce growth inhibition, apoptosis, and reduced invasive capacity through inhibition of molecules associated with tissue invasion such as uPA and MMP-9; (2) an anti-angiogenic activity, either direct, by affecting the proliferation and survival of endothelial cells, or indirect, by blocking the production of VEGF in MSC and in tumour cells; (3) an inhibition of osteoclast recruitment in bone lesions, by affecting the ability of bone marrow stromal cells to induce osteoclast differentiation and activation. All together, these findings highlight the importance of the activity of anti-EGFR agents on non-cancer cell types of the neoplastic microenvironment that might be involved in tumour growth and progression, such as endothelial cells and bone marrow stromal cells. Finally, the evidence summarized in this report strongly supports prospective clinical trials of anti-EGFR agents in cancer patients with bone metastases that will clarify the role of these drugs in the management of bone disease.

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