European Journal of Cancer (2013) xxx, xxx–xxx

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Evidence of epidermal growth factor receptor expression in uveal melanoma: Inhibition of epidermal growth factor-mediated signalling by Gefitinib and Cetuximab triggered antibody-dependent cellular cytotoxicity

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KEYWORDS
Epidermal growth factor receptor
Tyrosine kinase
Antibody-dependent cellular cytotoxicity
Targeted therapy

Abstract Despite advances in surgery and radiotherapy of uveal melanoma (UM), many patients develop distant metastases that poorly respond to therapy. Improved therapies for the metastatic disease are therefore urgently needed. Expression of the epidermal growth factor receptor (EGFR), a target of kinase inhibitors and humanised antibodies in use for several cancers, had been reported.

Forty-eight human UMs were analysed by expression profiling. Signalling was tested in three EGFR expressing UM cell lines by Western blotting using phosphorylation specific antibodies for EGFR and the downstream mediators AKT (v-akt murine thymoma viral oncogene homolog) and extracellular signal-regulated kinase (ERK). Evidence for signalling in tumours was obtained through the application of a UM-specific EGF-signature. The EGFR specific kinase inhibitor, Gefitinib and the humanised monoclonal antibody, Cetuximab, were tested for their effect on EGFR signalling. Natural killer cell mediated antibody-dependent cellular cytotoxicity (ADCC) and tumour necrosis factor α (TNF-α) release was analysed for Cetuximab.

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0959-8049/$ - see front matter
http://dx.doi.org/10.1016/j.ejca.2013.06.011

Please cite this article in press as: Amaro A. et al., Evidence of epidermal growth factor receptor expression in uveal melanoma: Inhibition of epidermal growth factor-mediated signalling by Gefitinib and Cetuximab triggered antibody-dependent cellular cytotoxicity, *Eur J Cancer* (2013), http://dx.doi.org/10.1016/j.ejca.2013.06.011

Fourteen of 48 UMs and three of 14 cell lines (over-)express EGFR, at least in part due to trisomy of the EGFR locus on chromosome 7p12. EGFR and the downstream mediator, AKT, are phosphorylated upon stimulation with EGF in EGFR expressing cell lines. EGFR over-expressing tumours but not EGFR negative tumours show an activated EGF-signature. Gefitinib inhibits EGFR and AKT phosphorylation and Cetuximab induces EGFR phosphorylation but inhibits signalling to AKT induced with EGF. Cetuximab triggers natural killer (NK) cells to lyse EGFR+ cell lines and to release TNF-α.
EGFR appears suited as a novel molecular drug target for therapy of uveal melanoma.

1. Introduction

Uveal melanoma, the most common primary intraocular malignant tumour in adults, arises from neoplastic proliferation of uveal melanocytes. Diagnosis of uveal melanoma is usually made by ophthalmoscopic examination and ultrasound. Uveal melanoma is molecularly clearly distinct from cutaneous melanoma (for a review see Ref.[1]). The recent identification of somatic mutations in uveal melanomas explains the molecular difference to cutaneous melanoma. BRAF mutations, frequent in cutaneous melanoma, were not detected in uveal melanomas that instead frequently carry mutations in GNAQ, GNA11 and BAP1.[2–4]

Several prognostic factors of disseminated relapse after initial ophthalmologic treatment have been determined, including cell morphology[5] location with respect to the equator,[6] retinal detachment,[6] monosomy 3[7] and additional cytogenetic markers,[8–10] syntenin protein expression[11] and a specific gene expression signature.[12] However, no effect of these prognostic markers on patient care can be envisaged in the absence of effective systemic therapies.

Treatment options are local radiotherapy (charged-particle beam therapy with protons or helium ions and the episcleral plaque radiation therapy) or enucleation. Uveal melanoma is defined by a poor natural outcome with a five year survival rate of 68.9%[13] that is considerably worse for patients with large tumours with monosomy of chromosome 3. Chemotherapy, such as oral temozolomide and intra-arterial fotemustine used at the metastatic stage, induces very low response rates, 4.3% and 36%, respectively, and a median survival time of 6.7 and 15 months.[14] No postoperative adjuvant therapies are currently available to decrease the risk of metastases. Treatment by systemic or intra-hepatic chemotherapy or partial hepatectomy only rarely prolongs life.[15]

The increased knowledge of molecular and genetic events associated with oncogenesis and tumour progression of ocular melanoma can lead to the identification of new therapeutic targets and agents. The preclinical investigation in relevant models is therefore mandatory to identify new therapeutic approaches. Many molecularly targeted drugs have been developed for other malignancies and if the responsiveness of uveal melanoma to these drugs can be shown, clinical trials could be designed in a very straightforward manner. We and others have therefore set out to identify targets of biological therapies. Hofmann et al. tested the tyrosine kinase inhibitor, Imatinib, that is specific for c-kit, a tyrosine kinase that is frequently over-expressed but not mutated in uveal melanoma. No objective response was observed.[16] Over-expression of the vascular endothelial growth factor (VEGF) has been reported[17] although its expression does not correlate with metastasis.[18] The basic fibroblast growth factor 2 (FGF2) and its receptor (FGFR1) have been described to activate the mitogen activated kinase, extracellular signal-regulated kinase 1 (ERK1).[19] Similarly, c-met, the receptor for the hepatocyte growth factor (HGF) has been shown to be frequently over-expressed by uveal melanomas[20,21] and its miRNA mediated inhibition can tame tumour cell proliferation and migration.[22] The insulin-like growth factor 1 (IGF1) and its receptor (IGF1R) have also been shown to be expressed in uveal melanoma[20,23,24] and to correlate with outcome.[20,24]

The recent identification of somatic mutations in the gene encoding the epidermal growth factor receptor (EGFR) in uveal melanoma cases indicates a potential etiopathological role for this tyrosine receptor kinase.[25] A mutated EGFR gene induces melanoma formation, including uveal melanomas, in transgenic fishes with 100% penetrance.[26] EGFR expression has been reported for uveal melanoma cell lines where it increases the ability of the cells to localise to the liver.[27] Hurks and coworkers described EGFR expression in human uveal melanoma and reported an inverse correlation with survival.[28] These data were challenged by a study that ascribed EGFR expression exclusively to tumour associated macrophages without addressing, however, the expression on uveal melanoma cell lines.[29] A more recent study reported EGFR expression on uveal melanomas using immunohistochemistry.[21]

In order to proceed to clinical trials, the efficacy of targeted therapies must be shown in appropriate cellular models. We present here an analysis of EGFR signalling in uveal melanoma cell lines and tumours and show the effects of the EGFR specific tyrosine kinase inhibitor, Gefitinib and the anti-EGFR monoclonal antibody, Cetuximab. Our data establish EGFR as a novel molecular drug target for therapy of uveal melanoma.

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