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Epidermal growth factor receptor expression in laryngeal cancer predicts the effect of hypoxia modification as an additive to accelerated radiotherapy in a randomised controlled trial ☆

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Abstract Accelerated radiotherapy (AR) improves the poor prognosis associated with epidermal growth factor receptor (EGFR) overexpression frequently seen in head and neck carcinomas. Combining AR with carbogen and nicotinamide (ARCON) counteracts enhanced tumour cell proliferation- and hypoxia-related radioresistance. The purpose of this study was to investigate if EGFR expression levels are associated with response to ARCON in patients with carcinoma of the larynx.

Patients ($N = 272$) with advanced stage larynx carcinoma were randomised between AR alone and ARCON. Paraffin-embedded biopsies from these patients were processed for immunohistochemical staining of EGFR. EGFR fraction was quantitated by automated image analysis and related to clinical outcome.

A large variation was observed in EGFR fraction between tumours with expression levels ranging from 0 to 0.93 (median fraction 0.4). No difference in 5-year locoregional control was found between low and high EGFR expressing tumours in the AR arm (69% versus 75%), which is in line with the established effect of AR in EGFR overexpressing tumours. There was, however, a significant association in the ARCON arm: patients with low EGFR

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levels had a better 5-year locoregional control (88% versus 72% $p = 0.02$) and disease-specific survival (92% versus 77% $p = 0.01$). ARCON improved locoregional control relative to AR only in patients with low EGFR expression (hazard ratio (HR) 0.34 $p = 0.009$).
In conclusion, only in tumours with a low EGFR fraction, adding hypoxia modification to AR has an additive beneficial effect on outcome. EGFR expression is a predictive biomarker for the selection of patients that will or will not respond to ARCON.

1. Introduction

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that plays a major role in regulating tumour cell proliferation and cell cycle progression.[1,2] EGFR is highly expressed in many solid cancers, including head and neck squamous cell carcinomas (HNSCC)[3] and is correlated with resistance to radiotherapy and decreased patient survival.[4] Ligand binding as well as ionising radiation can phosphorylate EGFR[5] leading to the activation of downstream cascades, like the phosphatidylinositol 3-kinase (PI3-K)/AKT and the mitogen-activated protein kinase (MAPK) pathways.[6] These signalling pathways are responsible for enhanced proliferation, cell cycle progression and increased DNA-repair leading to treatment failure.[2,7,8]

How well tumour cells respond to radiotherapy depends on their proliferative response induced by and during treatment, their ability to repair the radiation-induced DNA-damage and the amount of hypoxia within a tumour.[9] EGFR is involved in the regulation of intrinsic DNA-repair mechanisms and tumour cell proliferation via downstream pathway activation.[10] Tumour cell hypoxia induces radioresistance, directly as DNA-damage is maximised in the presence of oxygen and indirectly by promoting genetic instability.[11,12] An autocrine route has been described by which hypoxia induces the expression of EGFR and its ligands[13,14] and in addition, EGFR can stabilise one of the key proteins in the hypoxia response namely hypoxia-inducible factor 1α (HIF-1α).[11] Thus, EGFR is involved in all aspects of radioresistance. These resistance mechanisms play a role in HNSCC while EGFR is expressed at high levels in the majority of these tumours. This makes head and neck cancer the tumour archetype to further investigate these interactions.

Various studies have shown that enhanced tumour cell proliferation can be counteracted by accelerated radiotherapy (AR),[15–17] while tumour hypoxia can be reduced using hypoxia-modifying treatment modalities.[18] A strategy that combines both AR and hypoxia modification is accelerated radiotherapy with carbogen (98% O_2; 2% CO_2) and nicotinamide (ARCON).[19] Results from clinical trials with ARCON show high locoregional control rates, in particular for oropharynx and larynx tumours.[20,21]

Both irradiation[22] and hypoxia[13] can enhance the phosphorylation of EGFR thereby regulating intrinsic DNA-repair mechanisms[10] and cellular proliferation. We therefore hypothesised that EGFR could modulate the tumour response to accelerated radiotherapy with hypoxia modification. The purpose of our study is to investigate the predictive value of EGFR expression for ARCON in patients with advanced laryngeal carcinoma using material from a recently completed trial randomising between AR and ARCON.[21]

2. Materials and methods

2.1. Patients and treatment

Three-hundred-and-forty-five patients with advanced laryngeal carcinoma were included in a randomised trial comparing AR and ARCON between April 2001 and February 2008 at seven centres for head and neck oncology (six from the Netherlands and one from the United Kingdom (UK)). The eligibility criteria were published previously.[21] Approval from the local Ethics Committee of the Radboud University Nijmegen Medical Centre was obtained and all patients gave written informed consent. Pre-treatment paraffin-embedded biopsies were retrieved for immunohistochemical staining.

2.2. Immunohistochemistry

Sections from tumour biopsies were stained for EGFR expression as described previously with minor modifications.[23] Briefly, sections of 5 μm were cut, deparaffinised and rehydrated through a graded ethanol series. Sections were incubated with proteinase-K (DAKO, Glostrup, Denmark) at 37 °C. The primary antibody used was mouse anti-EGFR (DAKO M7239, Glostrup, Denmark) diluted 1:50 in PAD, primary antibody dilution (GeneTex Inc., USA). The secondary antibody was a biotinylated F(ab)′2-donkey anti-mouse immunoglobulin G (IgG) (Jackson Immunoresearch Laboratories Inc., West Grove, PA, United States of America (USA)), diluted 1:200 in phosphate buffered solution (PBS). Sections were counterstained with haematoxylin.