Critical Role of Tumour Marker Biology in the Assessment of Radiotherapy as a Therapeutic Modality in Oral Squamous Cell Carcinoma

**ABSTRACT** Despite advances in oncology therapeutics and biomedical research that have transformed since the turn of this century and deepened our understanding of cancer hallmarks, resulting in the discovery and development of targeted therapies, the success rates of oncology drug development remain low. Opportunities have increased with the understanding of molecular basis of the cancer translating to the discovery, development and availability of targeted therapies that have brought meaningful benefit to patients with diverse malignancies. One of the most challenging aspects of head and neck oncology is the selection of the most appropriate treatment for an individual patient with squamous cell carcinoma. The encouraging results from neoadjuvant chemotherapy and radiotherapy for patients with larger tumours, call for the identification of new accurate predictors of response to radiotherapy so that the appropriate therapy can be tailored better for individual patients.

**KEYWORDS** radiotherapy, squamous cell carcinoma, tumour markers

**INTRODUCTION**

The power of ionising radiation as a therapeutic modality relies in large part on two basic principles. One is the use of sophisticated delivery systems that maximises the dose delivered to the tumour as compared to the normal tissue, an approach epitomised by the advent of intensity-modulated radiation therapy. Intensity-modulated radiation therapy encompasses our way for sophisticated three-dimensional computer-based optimisation techniques and modern imaging techniques to devise flexible treatment plans that modulate the intensity of the radiation beams across a field, providing limitless possibilities to sculpt the radiation dose and avoiding excessive doses to critical normal tissues even if the tumour has an irregular shape. The second principle focuses on the finding that dose fractionation spares normal tissues with a slow turnover rate at the expense of tumours and normal tissues with a fast turnover. Radiation ‘tolerance’ doses are therefore largely determined by the amount that slowly proliferating tissues in the field can tolerate; a concept that is being partially revised in light of our ability to sculpt dose distributions. In any event, the concept has arisen that tissues with slow turnover ‘repair’ damage between dose fractions better than those with fast turnover, resulting in a therapeutic benefit, although this evidence is at the level of DNA repair and is annoyingly hard to obtain.

There have been several studies that suggest that immunohistochemical markers may play a prime role in the clinical management of patients with head and neck carcinoma by helping to predict which tumours are more likely to be eradicated by radiotherapy. Although most DNA repair proteins are expressed constitutively and in excess for the role they perform, the level of DNA damage and repair can influence intrinsic cellular radioresistance because cells with compact chromatin are more radiation-sensitive and it has been known for many decades that radiosensitivity is greater in the G2 and M phase of the cell cycle. The use of radiotherapy has been shown to improve the prognosis of various groups of oral cancer. On being used as the primary mode of treatment
it not only treats cancer in question but also treats the surrounding mucosal field in the oral cavity. If however, the tumour does not respond to radiotherapy, then not only is the prognosis worsened but the patient is also left with unpleasant side effects from the radiotherapy itself, with no benefit. Being able to predict this response to radiotherapy before treatment would improve prognosis and avoid unwanted side effects for these patients.

With the advance in modern molecular research techniques the mechanisms by which radiotherapy causes cell death have become clearer. The mechanisms by which tumour cells become radioresistant are complex, and the exact molecular pathways remain to be elucidated. Many factors exist which act at different phases of the cell cycle changing the susceptibility to radiotherapy. As things have moved on, immunohistochemistry procedures incorporating various factors such as microvessel density (MVD), molecular determinants including the role of p53, DNA-protein kinases, vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), Bcl-2 family and epidermal growth factor receptor (EGFR) have been investigated using techniques to determine the response to radiotherapy.

Role of MVD

For a tumour to grow and survive past approximately 1 mm³ it must induce a blood supply to gain oxygen and nutrients. Thus angiogenesis plays an important role in the development of a tumour. Conversely, hypoxia is thought to be one of the major causes of radiotherapy failure as the formation of oxygen free radicals is how the DNA is damaged⁴. So, increased angiogenesis can be both a poor prognostic factor as well as potentially being responsible for improving the chances for successful radiotherapy. One of the simplest and most widely accepted ways of assessing angiogenesis is to look at MVD i.e. to examine how many new vessels have been formed. Martin et al. (1997) in their research work assessed which of the three commonly used monoclonal antibodies; FVIIIRAg, CD31 and CD34 provides the best visualisation of microvessels in invasive breast cancer and used methods that gave reproducible data for the optimum number of ‘hotspots’ to count for each reagent. On analysis, the monoclonal antibody to CD31 was the least reliable antibody, immunohistochemically staining only 87% of sections compared with 98% for the monoclonal to CD34 and 99% for the monoclonal to FVIIIRAg. There was a high degree of correlation between the number of blood vessels stained by the different antibodies. Thus, the monoclonal antibody to FVIIIRAg was found to be the most reliable antibody. On the basis of the previous studies and literature, FVIIIRAg was selected to visualise the blood vessels for the intratumoral microvessel count and its luminal analysis⁷.

Brun et al. (2001) looked at 39 cases of oral squamous cell carcinoma (SCC) which all underwent preoperative radiotherapy (50 Gy) and assessed the MVD both before and after radiotherapy by staining with antibodies to FVIIIRAg. Although a high degree of MVD correlated with a poor prognosis, it had no relation with a response to radiotherapy⁸. Shintani et al. (2000) studied 41 cases of oral squamous cell carcinoma (OSCC) again with regard to MVD before and after radiotherapy, and found that MVD decreased after radiotherapy⁹. In the largest study of its type Aebersold et al., (2000) studied a population of 100 patients with OSCC all of whom received radical radiotherapy with a median dose of 74 Gy. Intratumoural MVD was identified by staining with antibodies to CD31. This study found that MVD did predict for response to radiotherapy (p = 0.01)¹⁰.

These studies have given disparate results in terms of predicting the response of a tumour to radiotherapy. A plethora of reasons may be responsible for this including the number of subjects in each study, amount of radiotherapy, stage of the tumours in the study and the combined use of other treatment modalities (chemotherapy and surgery) all are likely to deviate the picture. Whilst Brun et al. (2001) and Shintani et al. (2000) used a definition which effectively excluded any late recurrences by only looking at the initial response to radiotherapy Aebersold et al. (2000) used a more lenient definition which allowed for any recurrence be it early, late or at a distant site. Thus, MVD has an astonishing role in predicting radioresistance.

Role of p53

The p53 gene is one of the most commonly occurring mutated genes in cancer. The p53 gene encodes a nuclear protein which is involved in many cellular processes such as apoptosis, DNA synthesis and gene transcription. When a cell DNA is damaged by ionising radiation this damage is detected and the level of p53 protein is stabilised. The cell cycle is paused and the resultant response determines whether the cell enters into apoptosis or a DNA repair/survival pathway¹¹. A cell may, however, undergo p53 independent apoptosis if the dose of radiotherapy is high enough and DNA is sufficiently damaged¹². Many studies utilise immunohistochemistry as a method to detect the presence of mutated p53. This method assumes that very occasional positive staining of tumour cells correlates with an accumulation of wild type (WT) p53 in response to DNA damage while intense staining of most cells is due to mutation of the gene itself as p53 has a much longer half-life than the normal counterpart that is usually undetectable¹⁰. Jayasurya et al. (2004) concluded that an increased staining for p53 predicted for radioresistance (p = 0.002)¹². So it seems that the timing of radiotherapy as well as the other choice of treatment has a direct effect on the predictive value of p53.

DNA protein kinases

The DNA-protein kinase complex (DNA-PK) is involved in one of the major pathways by which a cell responds
to DNA double-strand breaks induced by ionising radiation. It consists of a heterodimer comprising 70- and 80 kDa proteins termed Ku and a 465 kDa serine/threonine protein kinase catalytic subunit termed DNA-PKcs. The Ku component functions as an activator of the catalytic subunit, and also represents the major double-stranded DNA-binding protein. As such it would be expected that the over or under expression of proteins that make up this complex could have some predictive value with regard to the response to radiotherapy. Shintani et al. (2003) performed a study on 42 human oral SCC specimens, all of whom had received pre-operative radiotherapy. An immunohistochemical analysis was performed with antibodies against DNA-PK complex proteins. Although the study found an increase in the expression of DNA-PK complex proteins after radiotherapy, there was no relationship between response to radiotherapy and DNA-PK complex proteins.

Vascular endothelial growth factor (VEGF)

Angiogenesis plays a major role in the development of radioresistance in many types of cancer, and a link has already been established in cervical and breast cancer. VEGF encompasses a family of proteins which importantly promotes new vessel growth and formation. They act via receptors principally found on the vascular endothelium. VEGF causes an upregulation of the antiapoptotic protein Bcl-2 thus protecting cells from the effects of radiotherapy. Studies have attempted to correlate the levels of VEGF with the outcome of radiotherapy. It has been concluded from previous studies that VEGF was up-regulated in all the tumours surviving radiotherapies as the levels of VEGF in the post-radiotherapy specimens were increased. The authors hypothesised that the radioresistant tumour cells had the potential to up-regulate VEGF in response to DNA damage. Smith et al. (2000) studied a total of 56 patients treated with a combination of surgery and post operative radiotherapy (to a median dose of 60 Gy). Immunohistochemistry was performed on tissue sections from the primary tumour before therapy began. Any tumour at local, regional or distant sites was taken to be a recurrence and thus indicate a resistance to radiotherapy. However, they did analyse each site of recurrence separately. They found that increased staining for VEGF predicted any recurrence (p = 0.007) and also predicted for local recurrence (p = 0.04).

Cyclooxygenase-2 (COX-2)

Prostaglandins are synthesised from arachidonic acid, and the cyclooxygenase (COX) enzymes are essential for this process. There are two isoforms of this enzyme, conveniently named COX-1 and COX-2. Of the two isoforms, COX-2 is induced during pathological processes such as inflammation and cancer including that of the head and neck region. One study proposes the COX-2 expression in histological biopsy specimens of oral SCC. In this study, biopsy samples were taken from 41 patients with oral SCC both before radiotherapy and after surgery, and immunohistochemistry was used to evaluate the expression of COX-2. Radioresistance was determined from the surgical specimens by analysing tumour mass remaining after the course of radiotherapy i.e. if two-thirds or less of the tumour mass did not show a response to radiotherapy the tumour was deemed radioresistant. When this was combined with the expression of COX-2, 17 of these specimens had a high level of COX-2 expression and statistical analysis showed a significant relationship between the over expression of COX-2 and radioresistance (p = 0.047).

Bcl-2 family

These are a group of proteins that are intimately involved in programmed cell death and are best described as being in two groups, those which promote and those which inhibit cell death. Promoters include bax, bak, bcl-xS, bad and bid while inhibitors include bcl-2, bcl-xl and bcl-w. A number of radioresistance factors such as EGFR, p53, DNA-PK complex and COX-2 exert their effects by modulating the apoptotic process; however the role of Bcl-2 family in oral SCC studies exist on the prevalence and prognostic value, but studies relating the response to radiotherapy are still into question.

Epidermal growth factor receptor (EGFR)

The family of epidermal growth factor receptors (EGFRs) consists of four tyrosine kinase transmembrane receptors that lie at the heart of a multitude of cell processes including cell proliferation, angiogenesis, migration and apoptosis. The EGFR signalling network is highly complex and consists of many layers. At present, there are 10 different ligands known to activate this pathway in mammals, epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) being two of the more well known. When the pathway is activated in the majority of cases, the result is stimulation of cell growth. However, this network has many other effects on the cell and when the receptor is activated by its ligands e.g TGF-α, it can cause activation of the signal transduction molecule stat3 that in turn promotes a rise in the antiapoptotic gene products such as bcl-xl. The theory that blocking this receptor would enhance the effects of radiotherapy, by decreasing the anti-apoptotic proteins, has been tested on a number of occasions. Huang et al. (1999) performed a study on cell lines derived from human head and neck squamous cell carcinoma (HNSCC). The cell lines used in the study were from the floor of mouth, tonsil and facial epidermis. An antibody, C225, was used to inhibit the effects of EGFR, and the growth of all cancer cell lines was inhibited. The cells were then exposed to a standard regime of radiotherapy in combination with the C225.
antibody. It was found that C225 significantly enhanced the effect of radiotherapy (p = 0.001)\textsuperscript{31}.

Harari and Huang (2001) performed a study on cell lines developed from oral SCC, which induced tumours in mice\textsuperscript{8}. They used C225 combined with radiotherapy and assessed levels of VEGF using immunohistochemistry both before and after treatment. They found that the expression of VEGF was reduced after radiotherapy alone and was reduced further when combined with the effect of C225. A further study performed by a different research group using tumour bearing nude mice concluded that the production of angiogenic factors such as VEGF in response to radiotherapy was the major method of inducible radioresistance\textsuperscript{43}. The C225 antibody has been used in a number of phase one, two and three trials, under the trade name “Cetuximab”, treating a broad group of patients with all types of head and neck cancer\textsuperscript{44}. The trials also involved many other forms of cancer including lung and colorectal cancer.

**FUTURE TECHNIQUES**

All of the studies quoted in this review so far have studied the expression of a single gene or protein with varying degrees of success. The process of studying gene expression has been a time-consuming process in the past with individual genes having to be studied separately. In the last few years the advent of microarray technology has revolutionised this field, allowing a large number of genes to be studied simultaneously and comparisons to be made between two distinct groups of tumours or between normal and tumour tissue.

**CONCLUSION**

Radiotherapy has been, and continues to be, a major treatment modality used in the treatment of oral squamous cell carcinoma. The prognosis of oral cancer has failed to show significant improvement over the past three decades. Resistance of oral cancer cells to the effects of ionising radiation remains an obstacle to improvement in the prognosis of this type of cancer. Radiotherapy in itself has some unpleasant side effects and when a tumour fails to respond to radiotherapy the prognosis is worsened further. The overall aim of this review was the emphasis on the mechanism of radioresistance and attempt to identify markers that could predict the response to radiotherapy for better understanding the prognosis and its further treatment modality.

**REFERENCES**


