The eukaryotic initiation factor 4E (eIF4E) as a predictive biomarker in the negative margins of resected head and neck and oral cancers: Is there a role for tailoring adjuvant radiotherapy

Bindhu Joseph[1,*], Rekha V Kumar[2], Champaka G[3], Ashok Shenoy[4], Sabitha K. S[5]

[1,3]Associate Professor, [2,4,5]Professor, [1]Dept. of Radiotherapy, [2,3]Dept. of Pathology, [4]Dept. of Head & Neck Oncology, [5]Dept. of Oral Oncology, Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, India

***Corresponding Author: Bindhu Joseph**
Email: bindhu271@yahoo.com

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Abstract

Introduction: The eukaryotic translation initiation factor 4E (eIF4E) is a protein that plays a key role in the tumourigenesis and metastasis. eIF4E overexpression usually precede morphological changes. The amplification of this biomarker in the surgically negative margins of resected Head and Neck cancer patients is associated with early recurrence and failure in several earlier studies. This subset may benefit from adjuvant radiotherapy even in the absence of morphological risk factors. We evaluated the pattern of expression of eIF4E protein in the surgically negative margins of Head and Neck cancer patients and its role in the treatment and outcome.

Material and Methods: Twenty-nine patients with Oral and 14 patients with Head and Neck cancers having surgically negative margins were analyzed for the expression of eIF4E with relation to local failure and treatment outcome.

Results: There were 11 local failures with 9 deaths, all within 6 months documented. The overall median survival was 12 months. Notably patients who had received adjuvant radiotherapy had a 45% better chance of survival 29.5 months vs 13.5 months (p=0.79). The clinical features of size, grade and co-expression of p53 did not correlate significantly with eIF4E overexpression. The Oral cancer subset when individually analyzed; showed a similar pattern of outcome with 17% (5/29) failing locally. Patients who had received adjuvant radiotherapy fared better with a 30% higher chance of survival 30.8 months vs 15.7 months. In Head and Neck cancer patients the failure rate was 57.8% (8/14). Patients who received radiotherapy had a median survival of 15.43 months vs 10.85 months (p=0.28).

Conclusion: The overexpression of eIF4E in surgically negative margins of resected Head & Neck and oral cancers may predict a more unfavorable microenvironment at risk for early recurrence and mortality within 6 months. The addition of adjuvant radiotherapy or targeting the eIF4E pathway may benefit these patients.

Keywords: eIF4E, Predictive biomarkers, Head & neck cancer.

Introduction

The new era of cancer treatment is focused on identifying Preclinical Biomarkers that may predict the aggressiveness of individual tumours. Primary surgery remains the main modality of treatment in early stages of Oral and several subsites of Head and Neck cancer. The need for Adjuvant treatment till date is based on morphological risk factors. Radiation is the most effective method of addressing subclinical disease in the tumour bed and adjacent tissue. It has an established role and survival advantage when used in patients with positive tumour margins or evidence of regional nodal spread after surgery.[1,2] However, 30-40% of Oral and Head and Neck cancer patients fail locoregionally inspite of not having these high-risk features. A possible hypothesis for this is that there may be a subset of patients with aggressive tumours whose adjacent microenvironment has preclinical changes of malignancy that are not morphologically evident. The ability to detect these changes at a molecular level could potentially help to prevent disease recurrence by identifying a subset of patients that may benefit from adjuvant radiotherapy inspite of negative histological margins. They would need tailored radiation with larger CTV (clinical target volumes) and broader categorization of nodal areas at risk.

The eukaryotic translation initiation factor 4 E (eIF4E) is a protein that plays a central role in cell growth and proliferation. The overexpression of eIF4E translates to uncontrolled proliferation and malignant transformation of immortalized fibroblasts which in turn later leads to tumour recurrence and metastasis.[3,4] Clinically the overexpression of eIF4E has been linked with aggressive tumour types and potential for early metastatic progression in a variety of malignancies including Head and Neck cancers,[5-9] Breast,[10-14] Colon,[15] Bladder,[16,17] Cervix[18,19] and Lung.[20] Earlier studies have demonstrated that the overexpression of eIF4E in tumour free surgical margins of Head and Neck and Oral Cancers correlated with a pattern of early locoregional failure and reduced survival.[2,22] In our study we have attempted to analyse the predictive role of this new biomarker with a larger Cohort of patients undergoing radical surgery as their primary treatment.

Material and Methods

Study Design: The present clinical study recruited 29 patients with oral cancer and 14 with Head and Neck Malignancies who underwent radical surgery as their primary management (2014-2017). All patients enrolled in the study had a single primary tumour, no previous history of malignancy and had not received prior neoadjuvant treatment. This study was approved by the institutional review board.

Histopathological Assessment: Paraffin embedded tissue blocks from the surgical margins were made as usual

following surgical resection. Specimens with histologically negative margins were further processed for the expression of eIF4E by immunohistochemistry (IHC). The 5% cut off value was selected for evaluating IHC staining of basal layer of the resected margins. Positive eIF4E expression was defined by the presence of brown perinuclear cytoplasmic staining involving greater that 5% of cells in the Basal epithelial layer.

Statistical Analysis

The data on patient characteristics and their association with eIF4E protein overexpression in the surgical margins was evaluated with contingency tables and Fishers exact test. The local recurrence rate and survival related to the pattern of expression of eIF4E in the surgical margins were estimated using the Kaplan Meyer method and compared using the Log rank test.

Results

The clinical profile of the patients was analysed for characteristics that may predict a higher possibility of having eIF4E being positive beyond the gross tumour. The parameters evaluated include tumour subsite, histological grade, size of primary and the presence of nodal disease. The only clinical feature that showed a significant correlation was the presence of nodal disease on post-surgical evaluation (p=0.005). Twenty-nine patients with Oral cancer and 14 with Head and Neck malignancies with negative post-surgical margins were included in our study. Their clinical characteristics are given in Table 1 and Table 2

Table 1

Clinical Parameter	Number	Percentage (%)
Subsite(Oral cancer)	29	
Buccal mucosa	15	51.7
Gingiva buccal sulcus	8	27.6
Tongue	5	17.2
Hard palate	1	3.4
Size>3cm	14	48.3
Size<3cm	15	51.7
Grade		
I	25	86.2
II	2	6.9
III	2	6.9
P53		
Positive	19	65.5
Negative	10	34.5
Node		
Positive	19	65.5
Negative	23	79.3

Table 2

Clinical Parameter	Number	Percentage (%)
Subsite(Head and Neck)	14	
Vocal cord	7	50
Post cricoid	2	14.3
Pyriform sinus	3	21.4
Aryepiglottic fold	1	7.1
Maxillary sinus	1	7.1
Size>3cm	6	42.9
Size<3cm	8	57.1
Grade		
I	7	50
II	3	21.4
III	4	28.6
P53		
Positive	3	21.4
Negative	11	78.6
Node		
Positive	6	42.7
Negative	8	57.1

It was observed that smaller tumours with lower grades may still have a risk of an aggressive adjacent microenvironment (eIF4E positivity). The occurrence of eIF4E was independent of the presence or absence of p53 overexpression. however, it was significantly overexpressed in the surgical margins of patients with pathological evidence of nodal disease.

Clinical outcomes associated with eIF4E overexpression in the surgically negative margins in

Oral Cancer Patients

Our study evaluated 29 Oral cancer patients with margins showing eIF4E overexpression. A negative outcome in terms of locoregional failure and/or death was observed in 5(17.2%) patients. There were 4 deaths and within a period of 4 months. The addition of adjuvant Radiotherapy appeared to have a protective benefit and survival advantage. The median survival for patients receiving adjuvant radiotherapy was 30.8 months and without it was nearly 50% less 15.74 months (P 0.746). With a median follow up of 12 months the Proportion surviving who received adjuvant radiation was 84.7%.

Head and Neck Cancer Patients

Fourteen patients with Head and Neck cancer were evaluated in the study. The overexpression of eIF4E in the surgical margins appeared to contribute more strongly to a negative outcome; compared to the Oral cancer patients. Six patients (42.9%) had treatment failure.

There were 5 deaths with 2 patients dying within 4 months. A similar pattern of survival benefit was observed for patients receiving radiotherapy. Patients who received adjuvant radiotherapy had a median survival of 15.42 months vs 10.85 months for those who didn't. The Median follow up was 13.3 months and the proportion who survived with adjuvant radiotherapy was 71.4%.

The survival benefit provided by adjuvant radiotherapy in patients (Head and Neck and Oral cancers) is represented in Fig. 1

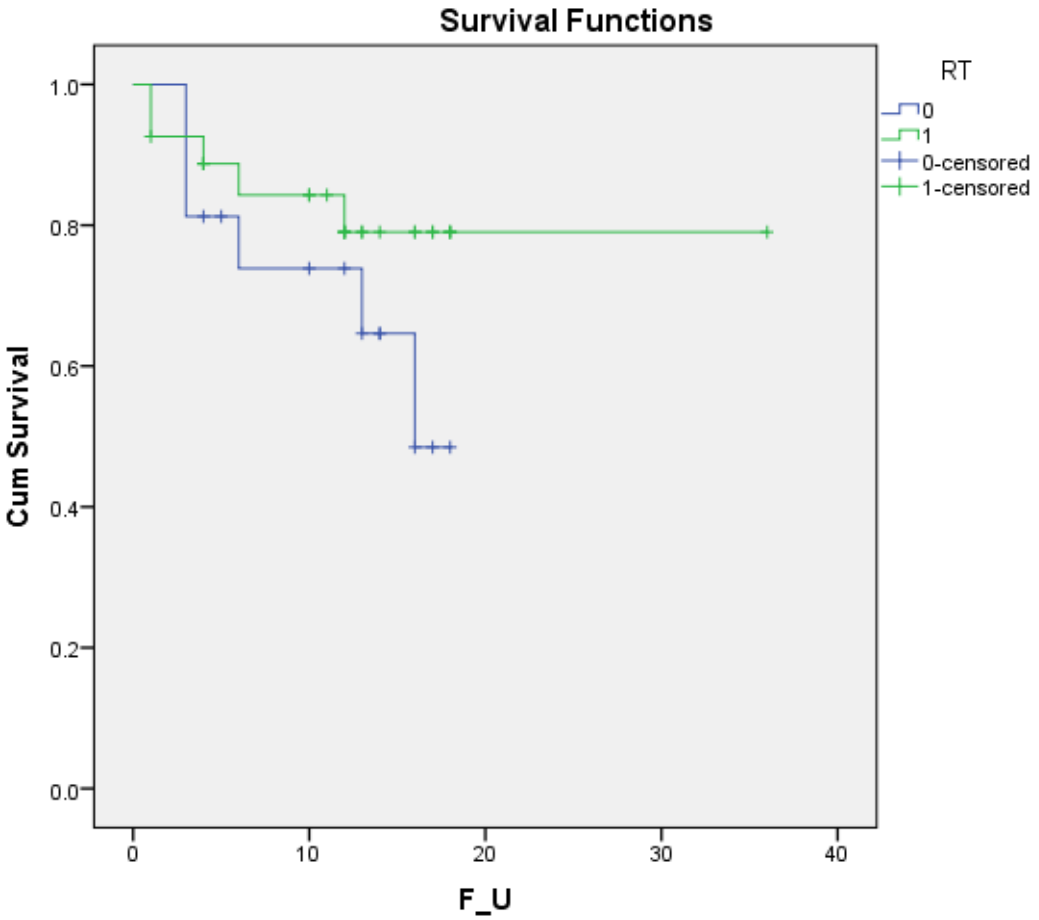

Fig. 1 (Original): Kaplan and Meyer recurrence curves of patients with eIF4E expression in their surgical margins who received adjuvant radiotherapy vs those who didn't

0-patients with adjuvant RT

1-patients with no adjuvant RT

When combining both cohorts of patients the pattern of benefit remained the same with patients receiving radiotherapy having a median survival of 29.5 months vs 13.5 months for those who didn't (P 0.23).

Discussion

The management of cancer is moving into an era of focused treatment tailored to the genetic and biological profile of individual tumour. The past few decades of cancer research has unearthed several predictive biomarkers and genetic patterns that may indicate the potential aggressiveness of a tumour in terms of its propensity for recurrence, metastasis and resistance to treatment. By identifying and targeting preclinical parameters of the oncological process in the paratumoural tissue there are chances of improving local control and survival.

The current treatment protocols for early stages of Oral and Head and Neck cancers involve radical surgery followed by adjuvant radiation or chemoradiation based on high risk post-surgical pathological features. The logic behind this approach is based on conceptual curves of therapeutic benefit derived from population-based survival models. The addition of adjuvant radiotherapy for positive margins and nodal involvement has resulted in proven benefit towards local control. However, inspite of this nearly a third of patients with Head and Neck cancer and more than 30% with Oral malignancies will recur.[2,22] A possible reason for is that, this approach fails to consider the biological heterogeneity of individual tumour and the preclinical changes of the oncogenic process in para-tumoural tissue that may be present inspite of negative surgical margins.

In our study we have evaluated eIF4E (Eukaryotic protein initiation factor) as a possible bio-indicator of tumour aggressiveness. The increase in eLF4E selectively promotes the translational efficacy of the transcripts CyclinD1, Pim-1m MMP9, FGF2, VEGF, c-myc and TLK1B which directly influence tumour growth, invasion, metastasis, angiogenesis and radio-resistance.[23] There is evidence to suggest that eIF4E overexpression may be an earlier molecular event than p53 mutation and is a more specific negative biomarker when detected in para-tumoural tissue.[24] A study conducted by Franklen et al. identified the elevation of eIF4E in surgical margins was an independent prognostic factor for poor outcomes. Several recent studies have demonstrated that overexpression of eIF4E in para-tumoural tissue of resected Head and Neck cancer patients put them at a higher risk for local recurrence inspite of pathologically negative margins.

Nathan et al reported a recurrence propensity of 42% (12/26) in patients of Head and Neck cancer who had elevated levels of eIF4E in their surgically negative margin. And notably, failure occurred at an average period of 3.4 months after surgery suggesting its potential role as a bioindicator of tumour aggressiveness.[25] A more recent study by Gulshan et al involving 26 patients with Head and Neck cancer demonstrated 8 patients with elevated eIF4E of which 7 (87.5%) had a recurrence. The median survival was 6.5 months in this cohort.[26]

In our study we have a larger study cohort of 43 patients. We included Oral cancer patients because radical surgery is the prime modality of management and there is a lesser role for upfront chemo-irradiation. The aggressiveness of eIF4E in this cohort of patients was not as evident as was for head and neck patients. A recurrence pattern of 5/29 (17.2%) was observed vs 6/14(42.9%) in the Head and Neck cancer patients. However, the propensity of early recurrence with majority occurring within 4 months of surgery remains similar to earlier studies.

An interesting result of our study was that adjuvant radiotherapy appeared to have a survival benefit in patients who expressed this negative biomarker in their surgical margins. In both cohorts of patients there was a near doubling of median survival time when radiotherapy was delivered as an adjuvant treatment. The same benefit also held true when both cohorts were analysed together. Patients who received radiotherapy had a median survival of 29.5 months vs 13.5 months in the subjects with no adjuvant treatment (P 0.23). YiLi et al evaluated the range of expression of eIF4E in terms of distance from the primary lesion in resected margins of 50 patients of oral cancer.[27] They observed a 30% chance of expression of this biomarker at 2 cm from the tumour which was significantly higher than for p53 and p21 CIP1/WAF1 which were also evaluated (P<0.005). In this study patients who had negative eIF4E at 2 cm from the tumour had a survival advantage over patients who had involvement at 0.5, 1 and 1.5cm respectively (p=0.004). These findings infer at the rationale for treating the para-tumoural microenvironment aggressively either in the form of re-excision to achieve biomarker negative margins or adjuvant radiotherapy with larger clinical target volumes (CTV) margins.

There are several ongoing pre-clinical strategies looking into targeting or subduing eIF4E activity.[28] One of these approaches involve testing Ribavaran; a competitive inhibitor of the natural ligand of eIF4E. It has shown some remarkable results in terms of clinical remission and blast response for patients suffering from refractory acute myeloid leukemia(French American British subtype – M4/M5).[29] These poor response leukemias are characterized by clinically detectable elevated levels of eIF4E. Thus, in patients with more advanced disease there may be a potential role for tailoring the chemo-irradiation protocol to match the aggressiveness of the clinical tumour scenario indicated by eIF4E.

Conclusion

The over expression of eIF4E in surgically negative margins of resected Head and Neck and Oral cancers predicts an unfavourable microenvironment as observed from the pattern of early recurrence and mortality within 6 months. This substantiates the fact that eIF4E has the potential to serve as a clinical biomarker of aggressive tumour behaviour even prior to morphological changes. It may identify a subset of patients who can benefit from adjuvant RT even in the presence of negative margins.

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