**Overexpression of cyclin A in oral dysplasia: An international comparison and literature review**

**Tandon R, Cunningham LL¹, White DK², Herford AS³, Cicciu M⁴**

Boyne Geistlich Research Fellow, ¹Department of Oral and Maxillofacial Surgery, Loma Linda University, Loma Linda, California, ²Departments of Oral and Maxillofacial Surgery and ³Oral and Maxillofacial Pathology, University of Kentucky, Kentucky, United States, ⁴Department of Human Pathology, School of Dentistry, University of Messina, Messina, Italy

**Correspondence to:** Prof. Marco Cicciù, E-mail: acromarco@yahoo.it

**Abstract**

Oral squamous cell carcinoma (OSCC) is one of the most debilitating cancers in the world and while its causes have been heavily researched, the outcome remains grim. Most of these cancers are identified in the late stage and as a result treatment options are limited. Therefore, researchers have focused their efforts on recognizing and identifying dysplastic tissue that has an increased chance of progressing to cancer. Research has begun to look at cell cycle dysfunctions and in particular, aberrant protein functions as a way of identifying the cellular mechanism at fault. The overexpression of a group of regulatory proteins called cyclins has been demonstrated in many types of dysplasia and carcinomas. Although researchers have identified several different types of cyclins as potential culprits, we chose to focus our study primarily on the overexpression of cyclin A. While most research on oral dysplasia and OSCC has been focused on cyclin D, studies have been done on cyclin A. While the etiology of oral dysplasia/SCC appears to be multifactorial, we chose to compare our results with those of similar studies performed across the globe. The social factors, such as the increased use of tobacco that may have contributed to our results, were compared with similar studies performed in Europe and Asia. While our results were remarkably similar and demonstrated a link between the overexpression of cyclin A in oral dysplasia, there exists some differences and thus may require a multicenter, longitudinal study.

**Key Words:** Cyclin A, oral dysplasia, oral cancer

**Introduction**

Oral squamous cell carcinoma (OSCC), a common head and neck neoplasm has proven to be one of the most malignant and lethal diseases in spite of modern and advanced therapies. This can prove to be a devastating phenomenon: As of 2009 there were 23,110 new cases of OSCC in the oral cavity and 7,566 new cases in the oropharynx. Of these new cases, 5,370 deaths occurred from those in the oral cavity and 1,338 deaths occurred from those in the oropharynx.¹ This poor survival rate is due to its silent behavior as most patients are not diagnosed until its advancement to an untreatable state. In recent years, treatment has focused on more preventative measures such as identifying clinically detectable pre-cancerous/neoplastic lesions. These lesions sometimes exhibit signs of oral epithelial dysplasia, which can progress to the development of SCC.

Dysplasia is characterized by cellular atypia, a loss of normal maturation and stratification while displaying no evidence of invasion.² Oral dysplastic lesions include leukoplaikia, erythroplakia and erythroleukoplaikia, which can exhibit an unpredictable rate of malignant transformation³⁴ that range from 0.3% to 36%. Oral dysplasia however can vary in intensity, which influences whether it progress to cancer or not. The higher grade of oral dysplasia, the higher the likelihood of cancer progression while the lower the grade, the lower the probability of progression to cancer and possibly even regression.⁵ Studies have demonstrated that dysplastic lesions that do undergo malignant transformations do so in a multi-step process.⁶ Unfortunately, this grading of dysplasia is prone to significant subjectivity and not a reliable predictor that the lesion will develop into carcinoma. Carcinogens such as tobacco and alcohol have the potential to induce alterations in genes that regulate the normal cell division, a topic that has gained widespread attention. Nevertheless, focus has been directed toward cellular events, in particular overexpression of proteins involved in the cell cycle as a means of identifying and subsequently, preventing this transformation. The goal of this paper is to discuss various studies identifying dysfunction of the cell cycle; specifically, the role of a group of proteins called cyclins as well as using our own data to support the conclusion drawn by other studies.

**Cell Cycle**

Normal cell division and proliferation is highly dependent on a series of complex and integrated events that must progress through the well-documented cell cycle. The cell cycle is controlled by a number of proteins referred to as the cyclins. Cyclins are a family of proteins that control the progress of cells through the cell cycle by activating cyclin dependent kinase (CDK) enzymes.⁹ There are two main groups of cyclins based on their function: G1 and mitotic.

The G1 cyclins include cyclins C, D1-D3 and E while the mitotic cyclins include cyclins A and B.¹⁰ The G1 cyclins regulate the passage of the cells through the G1 phase and their entry into the S phase while the mitotic cyclins facilitate the cell through the mitotic phase.

Cyclin D1 has been extensively studied as a facilitator of oral neoplastic lesions; its dysregulation, either through amplification and/or overexpression, has been shown to play an important role in precancerous oral epithelial dysplastic lesions and OSCC.¹¹⁻¹⁷ While cyclin D1 has been extensively studied, other cyclins such as cyclin A could also play an important role, either in conjunction or independently, in leading to oral dysplasia. Indeed, it has been demonstrated that cyclin A is a superior indicator of poor prognosis in colorectal cancer when compared with cyclin D.¹⁸ Therefore, a natural progression into the investigation of potential causes of oral dysplasia, which could lead to OSCC, would point to investigating the overexpression of cyclin A.
Cyclin A overexpression has been demonstrated in numerous tumors throughout the body: Colorectal,[18] esophageal,[19] non-small cell lung,[20] hepatocellular,[21] renal,[14] breast,[22] prostate,[23] and soft-tissue sarcomas.[24] Since cyclin A is produced in the S phase and present at the onset of deoxyribonucleic acid (DNA) synthesis, it is required for the cell to progress through the S phase and from G2 to mitosis. Phosphorylation of key components of the DNA replication pathway helps initiate the process. In this case, phosphorylation of cell division cycle 6 by cyclin A-CDK initiates DNA replication, but is normally restricted to once per cycle. After the cyclin A has performed its function, it is subsequently destroyed just before metaphase by ubiquitin-mediated proteolysis. With the importance of this function, it can be clearly seen how overexpression of cyclin A can cause increased cell growth, leading to potential tumor development.

Relatively few studies have been performed on cyclin A and its expression in dysplastic and cancerous tissue. The goal of this paper is to discuss those findings as well as use our results to support their conclusions. In addition, the demographics of our study differ considerably from many of the other studies as the patients from our study were natives of the Southeastern region of the United States (primarily central and Eastern Kentucky). While the results of each study demonstrated elevated levels of cyclin A, it is our opinion that there are underlying social factors that may play a role in this dysfunction.

Methods

A total of 41 specimens of previously diagnosed epithelial dysplasia were obtained from the Oral and Maxillofacial Pathology laboratory at the University of Kentucky College of Dentistry. The tissues were fixed in buffered formalin and then embedded in paraffin. All specimens were de-identified to protect patient privacy. A diagnosis provided and confirmed by two of the University of Kentucky Oral Pathology division faculty, was used for this evaluation. The sections were then deparaffinized with xylene and rehydrated with alcohol. Duplicate sections were overlaid onto glass slides prior to use.

Immunohistochemical staining was performed using antibody against cyclin A and the Avidin/Biotin protocol. Briefly, each section was prepared accordingly and hydrated using xylene for 5 min, 100% ethanol for 5 min, and then 95% ethanol for 5 min. After rinsing, the slides were placed in methyl hydrogen peroxide rinsed and exposed to the antigen retrieval solution (0.1 M citrate buffer) at boiling temperature for approximately 10 min and then simmered for 10-15 min. The slides were then cooled for 20-30 min at room temperature. Slides were then rinsed with several changes of deionized water and then placed in 1X Phosphate Buffered Saline PBS solution. The slides were then flooded with normal serum and placed in a humidity chamber into a 37°C oven for 10 min. After blotting primary antibody was applied (cyclin A specific antibodies, Santa Cruz Biotechnology, Inc. Santa Cruz, CA) and slides were incubated in a humidity chamber, at 37°C for 45 min. Slides were rinsed in PBS X2 solution and left to stand for 5 min. After blotting, the secondary antibody, which contained anti-rabbit immunoglobulin G antibody, was applied and slides were incubated at 37°C for 18 min. After rinsing, the Avidin/Biotin complex (ABC, Vector Laboratories, Burlingame, CA, USA) was applied in a humidity chamber for 18 min followed by ×2 PBS rinses and 3,3-Diaminobenzidine solution development for 5 min and distilled water rinses. Slides were counterstained with Harris’ hematoxylin, then dehydrated, cover-slipped and viewed.

Results and Statistical Analysis

A total of 37 dysplastic samples and 8 normal samples of oral squamous epithelium were available for final evaluation. Two independent investigators counted the stains of each sample and the results were then averaged between the two. In the dysplastic tissue, a mean of 39.3 cells/3 mm sample (95% Confidence Interval [CI]: 31-48) stained positive for cyclin A. Positively stained cells were found throughout the epithelial layers and ranged from mild to moderate to severe dysplasia. In the normal epithelium, a mean of 16.3 cells/3 mm sample (99% CI: 5-27) stained positive and were found in the basal and parabasal cell layers only. A rank sum analysis supported a statistical difference between the samples at P = 0.003. Using a threshold positive cut-off of 31 cells/3 mm, the frequency of positive specimens was significantly elevated in the dysplastic tissue (P = 0.0023).

A contingency analysis showed a positive predictive value of 70.3%, specificity of 38.1% and an Accuracy of 86.5% for this descriptive characteristic of dysplastic tissues.

Discussion

OSCC continues to be one of the world’s most lethal and destructive malignancies. Its predictability has eluded clinicians; thus, making it one of the most devastating carcinomas to treat in spite of advances in both surgery and radiotherapy.[25,26] Such gloomy projections have led investigators to look toward more preventative measures as a means of treating OSCC, namely identifying those dysplastic lesions that have a higher potential toward malignant transformation. Histological analysis of such lesions has shown to be effective in identifying the potential of such lesions, providing optimism to both clinicians and patients. While these new advances provide hope, there is still no definitive and accurate prognostic marker necessary to determine the outcome. Many human tumors are characterized by significant increases in cell proliferation and since cyclins play a key role in regulating the passage of dividing cells through the various check-points, investigation into their behavior is warranted.

Our study focused on determining the overexpression of cyclin A in dysplastic tissue as compared with normal tissue. We used an immunohistochemical analysis to label the protein in both sets of tissues, which were then independently counted by two separate investigators. The purpose of the investigation was to support previous studies regarding cyclin A’s effectiveness in predicting dysplasia. Thomson et al., performed a similar study and...
utilized a computer-assisted microscopy system to analyze the dysplastic and cancerous tissue after staining with immunohistochemistry.\(^{[27]}\) Whereas our assessment was based on identification and collaboration between two independent investigators, they utilized advances in technology to more objectively come to their conclusions. In spite of this difference in methodology, our results show similarities as cyclin A was elevated for moderate to severe dysplasia when compared with the mild variant and normal tissue.\(^{[27,28]}\)

Studies focusing solely on patient population in the continental United States have been limited and lacking in comparison to those found in other areas such as Europe and Asia. The results of Thomson's study, while supportive of our own results, were limited in that it was focused on patients in the United Kingdom. Similarly, Chen et al., demonstrated the high incidence of cyclin A overexpression in OSCC in Taiwan.\(^{[10]}\) While this study is often cited as proof of cyclin A's role in dysplastic progression and eventual tumor formation, the authors do in fact acknowledge the prevalent use of the areca nut, which is chewed in many southeastern Asian countries.\(^{[10,29]}\)

Although, the habit of chewing this nut is associated with a high incidence of oral cancer in India and other countries in the region, it sometimes lacks tobacco.\(^{[30]}\) This, however, is not the case in central and eastern Kentucky in the United States. In Kentucky, approximately 25.2% of the adult population is cigarette smokers and over 7,800 adults died as a result of tobacco use between 2000 and 2004, placing Kentucky at the bottom of tobacco mortality rate in the United States.\(^{[31]}\) Smokeless tobacco while not as prevalent, is a habit undertaken by nearly 6.7% of the adult population Kentucky.\(^{[31]}\) Likewise alcohol may also play a role in the progression of dysplasia, as Hamadah et al., found that nearly 75% of patients with precancerous lesions reported regular alcohol consumption.\(^{[31]}\) Nevertheless, there was virtually no correlation between the developments of single versus multiple lesions with the amount of alcohol consumed.

Many studies have been published on cyclin A's role in oral dysplasia throughout the world, but it has yet to be extensively studied here in North America. While our results correlate positively with studies done elsewhere, demographics and social behavior must be taken into consideration when assessing risk factors for oral dysplasia. We have been able to support other investigators around the world in their hypothesis that cyclin A is overexpressed in oral precancerous lesions. However, a more organized, multicenter, longitudinal study should be undertaken to confirm these results.

References

Occasionally, one sees edema of massive edema, suggesting that at least in some cases, fibromatosis areas in a lesion that consist predominantly in a lesion that is predominantly fibromatosis or some fibrous character simulating a solid fibroma rather than effaces the underlying normal ovarian structures in contrast to massive edema; however, it has a firm, white appearance. The stroma shows focal collection of chronic inflammatory cells infiltrate [Figure 1]. Final diagnosis of ovarian fibromatosis ovary was made.

We received two soft tissue pieces measuring 3 × 2 × 1 cm and 4 × 2 × 2 cm; outer surface smooth and pearly white (appear to be part of ovary).

Right ovary was grossly enlarged measuring 7 × 5 × 2 cm. The surface was smooth, firm in consistency, and at places free fluid in the peritoneal cavity. Bilateral tubes were grossly normal. Uterus was grossly enlarged to 10 weeks size. Exploratory laparotomy was done. There was about 50cc of free fluid in the peritoneal cavity. Because of the benign nature of the tumor.

A 27-year-old unmarried female presented with history of pain in lower abdomen and excessive bleeding during menstruation for 5 years. A 25-year-old patient presented with abdominal and pelvic pain, and occasionally hirsutism described by Young and Scully in young patients (mean age, 25 years) who present with menstrual abnormalities, ovarian fibromatosis characterized by diffuse ovarian fibrosis is a rare benign disorder first perceived at laparotomy and to allow conservative treatment. Correct pretreatment diagnosis is crucial to determine a ovarian neoplasm. Ovarian fibromatosis is closely related to ovarian edema, fibromas, Brenner, and Krukenberg's tumor.

The case highlights the importance of recognizing a rarer lesion that differs from other pelvic fibrotic process such as fibroma, Brenner tumor, massive edema, and virilization. Ovarian fibromatosis is closely related to ovarian edema, fibromas, Brenner, and Krukenberg's tumor.

References


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