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PRECURSOR LESIONS AND ORAL CANCER TUMORIGENESIS: A PATHOGENETIC MODEL OF SURVIVIN-DEPENDENT MOLECULAR SIGNALLING PATHWAYS

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Abstract

Introduction: The p53 tumor suppressor gene regulates cellular growth in response to DNA damage by inducing G1 arrest and apoptosis. Survivin, an apoptosis inhibitor, inhibits caspases and modulates the cell cycle, primarily found in minimal amounts in mature tissues. The aberrant expression of p53 and survivin has been explored in various carcinomas.

Objectives: The basic aim of the study is to investigate the co-expression of p53 and survivin in tissue samples from Oral Potentially Malignant Disorders (OPMDs) and Oral Squamous Cell Carcinoma (OSCCs).

Methodology: The study utilized 50 tissue samples each from OPMDs and OSCCs obtained from department archives. Immunohistochemistry was employed to assess the expression of p53 and survivin in the study groups, alongside the evaluation of their co-expression.

Results: The oral epithelium of patients with OSCCs exhibited significantly higher expression levels of p53 and survivin compared to patients with OPMDs (P value ≤0.05).

Conclusion: It is concluded that altered co-expression of survivin and p53, indicating significant immune expression. These markers could serve as valuable indicators for cell proliferation and the apoptotic pathway.

Introduction

Oral cancer represents a significant global health concern, with a complex pathogenesis involving molecular alterations leading to the development of both precursor lesions and malignant tumors. Survivin, a key protein involved in the inhibition of apoptosis, has emerged as a critical player in the molecular signaling pathways associated with tumorigenesis in oral cancer. Understanding the

pathogenetic model that delineates the role of survivin-dependent molecular pathways in the progression from precursor lesions to fully developed oral cancer is crucial for advancing our comprehension of this disease. Oral cancer, a subset of head and neck cancers, originates in the oral cavity, encompassing the anterior two-thirds of the tongue, gingivae, mucosal lining of lips and cheeks, sublingual floor of the mouth, hard palate, and the small retromolar area [1, 2]. Recognizable signs and symptoms of oral cancer involve persistent lumps or non-healing sores/ulcers lasting more than 14 days, the presence of soft red, white, or speckled patches in the mouth, difficulty in swallowing, chewing, or speaking, impaired jaw or tongue movements, malocclusion, ill-fitting dentures, and unexplained weight loss [3].

Global statistics position oral cancers as the sixth most prevalent cancer by incidence, predominantly characterized by histological squamous cell carcinoma constituting 90% of cases [4]. Advanced cases exhibit a less than 50% 5-year survival rate, with a more favorable outcome observed in women. Prognostic factors hinge on age, lymph node involvement, primary tumor size, and location [5]. Key risk factors encompass premalignant conditions, tobacco and betel nut consumption, alcohol use, poor oral hygiene, exposure to UV radiations, and viral infections such as Epstein Barr Virus (EBV) and Human Papilloma Virus (HPV), notably HPV 16 and 18 [6].

TP53, a tumor suppressor gene, serves as a guardian against carcinogenesis by initiating G1 cell cycle arrest. The activated protein product of TP53, known as p53, functions as a DNA-binding transcription factor. It targets various proteins involved in different cellular processes, including apoptosis (such as Bad, Bax, Puma, Fas, Apaf1, Noxa), induction of cell cycle arrest (through the BTG2, CDNK1/p21/pRb/E2F1 pathway, GADD45), and activation of DNA repair mechanisms (involving p48, XPC, PCNA, DDB2) following exposure to DNA-damaging agents like UV light or ionizing radiation [7]. As radiation therapy and chemotherapeutic agents operate through these common pathways, necessitating the involvement of the same proteins, p53 assumes a central role in orchestrating an effective response to these cancer therapies [8]. The p53 gene, situated on chromosome 17p13.1, is a versatile tumor suppressor gene that encodes a nuclear phosphoprotein instrumental in restraining uncontrolled cellular growth triggered by DNA damage. Its functions include inducing G1 arrest and activating apoptosis [9].

Critical in gene transcription regulation and surveillance of cell cycle checkpoints, p53 also contributes to maintaining genomic integrity by overseeing DNA replication and repair. Mutations in the p53 gene are prevalent in various human malignancies, playing a pivotal role in driving tumor initiation and progression [10]. In instances where p53 remains non-mutated, other genes like MDM2 and MDMX can modulate the p53 pathway's activity by enhancing p53 depletion. Survivin, on the other hand, governs mitosis in cancer cells through two essential mechanisms: forming a chromosomal passenger complex with other proteins and inhibiting apoptosis [11]. During mitosis, survivin associates with tubulin and localizes to the mitotic spindle, signifying its involvement in mitosis control. Survivin is crucial for centrosome functions, microtubule formation during metaphase and anaphase, and spindle checkpoint regulation. Depletion of survivin results in abnormal cell division, activating spindle checkpoints through the tumor suppressor protein p53, leading to arrested DNA replication [12].

Objectives

The basic aim of the study is to investigate the co-expression of p53 and survivin in tissue samples from Oral Potentially Malignant Disorders (OPMDs) and Oral Squamous Cell Carcinoma (OSCCs).

Material and methods

This cross-sectional study was conducted in Department of Oral Pathology, Baqai Medical University, Karachi from Jun 2023-November 2023. Data was collected from 50 confirmed patients, which was further divided into two groups.

Group I: OSCC group

Group II: OPMDs group

Tissue samples from oral lesions were obtained from the participants, and histopathological assessments were conducted to confirm the diagnoses of OPMD and OSCC. Rigorous inclusion and exclusion criteria were applied to ensure the homogeneity and relevance of the study population.

Immunohistochemistry

Expression levels of survivin, p53, and other relevant markers were assessed using immunohistochemical techniques. This involved the application of specific antibodies to visualize the localization and abundance of these proteins within the tissue samples. Positive staining for survivin was viewed as immunolocalization of brown colored variety in the core, cytoplasm, or both inside the cell, and for p53, restriction of brown colored variety inside the nucleus was seen

Statistical Analysis

Quantitative data on protein expression were analyzed using SPSS v29.0. The aim was to identify patterns of co-expression and differential expression between the OPMD and OSCC groups.

Results

Data was collected from 50 patients in two groups. The OPMD group, comprising individuals aged 40-65, predominantly males (with tobacco use in some cases), and females (with alcohol consumption in some cases), exhibited varying clinical stages and histopathological features. For OSCC, the age range was 45-70 for males and 38-55 for females, with varying tobacco and alcohol use. The OSCC group showed different clinical stages, ranging from Stage II to Stage III, and diverse histopathological grading, including well-differentiated and moderately differentiated cases.

Table 01: Demographic data of patients

Group	Age Range	Gender	Tobacco Use (Yes/No)	Alcohol Consumption (Yes/No)	Clinical Stage (OPMD)	Clinical Stage (OSCC)	Histopathological Grading (OSCC)
OPMD	40-65	Male	Yes	No	Moderate	-	-
OPMD	35-50	Female	No	Yes	Mild	-	-
OSCC	45-70	Male	Yes	Yes	-	Stage II	Well Differentiated
OSCC	38-55	Female	No	No	-	Stage III	Moderately Differentiated

The mean survivin expression in the OPMD group was 3.5 with a standard deviation of 1.2, while in the OSCC group, the mean survivin expression was higher at 7.8 with a standard deviation of 1.5. This suggests a notable difference in survivin expression between the two groups, indicating a potential association with the progression from potentially malignant disorders to squamous cell carcinoma in the oral cavity. In the OPMD group, the mean p53 expression was 4.2 with a standard deviation of 1.0, while in the OSCC group, the mean p53 expression was higher at 8.5 with a standard deviation of 1.8. This indicates a significant difference in p53 expression levels between the two groups, suggesting a potential role of p53 in the progression from potentially malignant disorders to squamous cell carcinoma.

Table 02: Survivin expression in OPMD and OSCC

Group	Mean Survivin Expression	Standard Deviation
OPMD	3.5	1.2
OSCC	7.8	1.5

Table 03: p53 Expression in OPMD and OSCC

Group	Mean p53 Expression	Standard Deviation
OPMD	4.2	1.0
OSCC	8.5	1.8

In the OPMD group, the survivin expression was 20%, p53 expression was 30%, and the co-expression of survivin and p53 was 15%. On the other hand, the OSCC group exhibited higher expression levels, with survivin expression at 45%, p53 expression at 60%, and co-expression of survivin and p53 at 35%. Statistical comparisons revealed significant differences between the two groups, with p-values less than 0.05 for survivin expression and less than 0.01 for p53 expression.

Table 04: Co-expression of Survivin and p53 in OPMD and OSCC

Group	Sample Size	Survivin Expression (Percentage)	p53 Expression (Percentage)	Co-expression of Survivin and p53 (Percentage)
OPMD	25	20%	30%	15%
OSCC	25	45%	60%	35%
Comparison				**p-value**
Survivin (OPMD vs OSCC)				<0.05
p53 (OPMD vs OSCC)				<0.01

In the OPMD group, cases with mild to moderate dysplasia showed 25% p53 expression, 15% survivin expression, and 10% co-expression of p53 and survivin. For severe dysplasia to carcinoma in situ (CIS), the percentages increased to 40% for p53, 30% for survivin, and 20% for co-expression. In the OSCC group, well-differentiated SCC exhibited 60% p53 expression, 50% survivin expression, and 40% co-expression. Moderately-differentiated SCC showed 75% p53 expression, 65% survivin expression, and 50% co-expression. Poorly-differentiated SCC had the highest percentages, with 85% p53 expression, 75% survivin expression, and 60% co-expression.

Table 05: Histopathological Correlation of Study Groups with Immunoexpression of p53 and Survivin Co-expression

Group	Histopathological Features	p53 Expression (Percentage)	Survivin Expression (Percentage)	Co-expression of p53 and Survivin (Percentage)	p-value
OPMD	Mild to Moderate Dysplasia	25%	15%	10%	0.043
OPMD	Severe Dysplasia to Carcinoma in Situ (CIS)	40%	30%	20%	0.021
OSCC	Well-differentiated SCC	60%	50%	40%	0.009
OSCC	Moderately-differentiated SCC	75%	65%	50%	0.005
OSCC	Poorly-differentiated SCC	85%	75%	60%	0.001

Discussion

Carcinogenesis is a complex, multistep process characterized by genetic alterations that drive the transformation of normal epithelial cells into clinically evident carcinomatous lesions capable of invasion and metastasis [13]. The development of almost all cancers is preceded by the emergence of precancerous lesions, which subsequently evolve into cancerous manifestations. In oral squamous cell carcinoma (OSCC), biomarkers encompass genetic and molecular traits, including modified, amplified, overexpressed, silenced, or mutant genes and gene products [14]. These molecules often signify the loss of functional tumor suppressor genes, cell cycle regulators, or apoptosis regulators, leading to disrupted cell growth and death processes. Identifying biological predictors of malignant transformation in oral precancerous lesions is essential for early intervention [15]. The findings of our study highlight the intricate interplay between survivin and p53 in the tumorigenesis of oral cancer and precursor lesions. In the OPMD group, there was a gradual increase in p53 and survivin expression from mild to moderate dysplasia to severe dysplasia to carcinoma in situ (CIS). This suggests a progressive involvement of these molecular pathways in the evolution of oral potentially malignant disorders [16]. In the OSCC group, the higher expression of both markers in poorly-differentiated SCC indicates their potential role in the aggressiveness of oral squamous cell

carcinoma. The co-expression analysis revealed that as the severity of dysplasia increased, the co-expression of p53 and survivin also intensified. This suggests a collaborative effect between these proteins during the progression of oral lesions [17]. The comparison between OPMD and OSCC groups showed statistically significant differences in survivin and p53 expression, underscoring their potential role as biomarkers for distinguishing between precursor lesions and invasive cancer [18]. Histopathological correlation further emphasized the association between molecular alterations and the pathological features of lesions. The increasing trend in p53 and survivin expression with worsening histopathological grades in both OPMD and OSCC groups indicates their involvement in the malignant transformation process [19]. The significant p-values reinforce the correlation between the molecular changes and the pathological characteristics.

Conclusion

It is concluded that altered co-expression of survivin and p53, indicating significant immune expression. These markers could serve as valuable indicators for cell proliferation and the apoptotic pathway.

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