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ERCC1 and MutS Expression in Ameloblastoma-An Immunohistochemical Study

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Abstract

Background: Although it is benign, it is locally aggressive and the factors which are favoring this are not fully understood. One of the proposed models of the evolution of ameloblastoma is related to alteration in DNA damage & repair mechanism. Thus identifying a faulty DNA repair mechanism could be advantageous in planning and implementing the treatment protocol.

ERCC1 and MutS are the DNA repair proteins that help in reducing mutations & maintaining genomic stability. Ample studies have been done on ERCC1 and MutS expression in several malignancies. However, there is subtle research focusing on ERCC1 and MutS expression in odontogenic tumors. The present study is done to fill this lacuna.

A better understanding of DNA repair mechanisms and molecular pathogenesis in ameloblastoma may assist in improving diagnostic methods & predict the treatment outcome for a given patient.

To evaluate the expression of ERCC1, MutS in Ameloblastoma.

Method: Tissue blocks of prediagnosed cases of Ameloblastoma were collected from the archives of the Department of Oral Pathology, St. Joseph Dental College, 3 to 4-micron thin sections were prepared from the retrieved blocks & subjected to the immunohistochemical staining procedure. Using ERCC1 & MutS immunohistochemical (IHC) markers. The stained slides were analyzed under a Trinocular Olympus Bx53 Progress CT research microscope. The results were tabulated & subjected to statistical analysis

Conclusion: In conclusion, the development of an effective non-invasive treatment for ameloblastoma has been hampered by a delayed and insufficient diagnosis. A

better understanding of DNA repair mechanisms & molecular pathogenesis in ameloblastoma may assist in improving diagnostic methods and to deliver the accurate treatment for a given patient.

Keywords: DNA Repair Mechanisms, ERCC1, MutS, Immunohistochemical.

Introduction

According to WHO, Ameloblastoma is defined as a "true neoplasm of enamel organ type that does not undergo differentiation to the point of enamel formation" (Or) In 1937 ROBINSON defined Ameloblastoma as a benign tumor that is usually "unicentric, non-functional, intermittent in growth, anatomically benign and clinically persistent." [1]

Ameloblastoma pathogenesis and progression have been associated with mutation in *p53* and related proteins (p63, p73), increase in gene/protein expression of p53 and MDM2 (a negative regulator of tumor suppressor p53), and survivin (involved in the progression of cell cycle and inhibit apoptosis) or degradation of p53 protein. Sneh M Toprani et.al. proposed one of the models of the evolution of Ameloblastoma is related to alteration in DNA damage & its repair mechanisms. Numerous factors can cause DNA damage, which can be repaired with the aid of DNA repair mechanisms.

The growth, progression & survival of tumors depends on the DNA repair mechanism. ERCC1 & MutS are the two DNA repair proteins that help in reducing mutations & maintaining genomic stability.[2] MutS is a key enzyme in DNA mismatch repair that corrects mismatched bases, produced during DNA replication & other biological processes. Deficiencies in the MutS protein disrupt methyl-directed mismatch repair (MMR) generating a mutator. ERCC1 (Excision Repair Cross-Complementation Group-1) plays an essential role in the removal of DNA intrastrand crosslinks by nucleotide excision repair. The objective of the present study is to compare the immunohistochemical expression of ERCC1 & MutS (DNA repair proteins) between ameloblastoma & normal tissues. Identifying a DNA repair biomarker could be advantageous to eliminate the tumor[2].

The main aim of this research is to evaluate the ERCC1 and MutS Expression in Ameloblastoma.

The objective of this study is to compare the expression of ERCC1, and MutS expression in ameloblastoma and normal tissues.

Materials and Methodology

Fifteen tissue blocks of histopathologically identified Ameloblastoma and normal oral mucosa were retrieved from the oral pathology department's archives at St. Joseph Dental College in Eluru. 3 to 4-micron thick tissue sections from each tissue block were taken on positively charged slides using a semiautomatic microtome (Thermo scientific Microm HM 340E, Novel technologies). Slides were incubated for 1 hour & then deparaffinized three times for five minutes each in xylene and then rehydrated in descending grades of alcohol. Antigen retrieval was done using Tris-EDTA buffer in a pressure cooker for 15–20 minutes until pressure was released automatically through 2 whistles. Endogenous peroxidase was blocked by incubating the slides with peroxide blocking reagent for 5 min. After washing with Tris buffer solution (TBS), the sections were incubated separately with ERCC1 and MutS antibody**.** The sections were then treated with Linker 1 (HRP) for 5 minutes at room temperature followed by Linker 2 (Streptavidin biotin) for 5 minutes at room temperature. The reaction products were then visualized using DAB chromogen, incubated for 2 minutes at room temperature, and counterstained with Harris hematoxylin. The slides were then analyzed under a Trinocular Olympus Bx53 Progress CT research microscope.

Microphotographs of all the IHC stained sections under 40× magnification were taken using Prog Res R Capture Pro 2.8.8 JENOPTIK. Five non-overlapping fields of ameloblastic islands with uniform staining were randomly selected for each slide for imaging. In each field of island, the total number of cells and positively and negatively stained cells were counted using Image J software. H scores were calculated from the data obtained. Based on IHC staining intensity, the cells of each selected field were categorized into type 1 cells: unstained cells: type 2 cells: bluish brown stain; type 3 cells: faint brown stain; type 4 cells: dark brown stain [Figures 1 and 2].

Type1 cells: unstained cells

Type 2 cells: bluish-brown stain

Type 3 cells: faint brown stain

Type 4 cells: dark brown stain

Where the score for –

The intensity of type 1 cells is 0

The intensity of type 2 cells is 1

The intensity of type 3 cells is 2

The intensity of type 4 cells is 3

The average H score of 5 fields of each slide was considered as the final H score of that slide.

H score derivation for ERCC1 & Mut S expression was done by using the following formula.

H Score = [(1+intensity of type1 cells) x total % of type 1 cells) + (1+intensity of type 2 cells) x total % of type 2 cells) + (1+intensity of type 3 cells) x total % of type 3 cells) + (1+intensity of type 4 cells) x total % of type 4 cells)][6].

Result

The mean H score of Mut S expression in Ameloblastoma and in normal tissues by using Mann Whitney U test was 284.55 ± 76.64 (mean ± SD) & 237.10 ± 26.08 (mean ± SD) respectively as indicated in Table 1 & graph 1.ERCC1 expression in normal tissues by using Mann Whitney U test was 171.47± 37.044 (mean ± SD) as indicated in table 2 & graph 2.

Figure 1 indicates a positive expression of MutS in ameloblastoma cells & positive expression of ERCC1 in ameloblastoma cells.

Inference

1. The results elaborated in table 1 & graph 1 indicate a significantly higher MutS expression in ameloblastoma compared to normal tissue.
2. The results elaborated in table 2 & graph 2 indicate positive expression of ERCC1 in ameloblastoma to normal tissue.

Table 1: comparison of positive cells among the normal tissue and Ameloblastoma (Mut S)

Groups	Mean	Std. Deviation	Mean ranks	Mean diff	Uvalue	Pvalue
Normal tissues	237.10	26.08	9.00	47.44	35.00	0.003*
AMELOBLASTOMA	284.55	76.64	18.75			

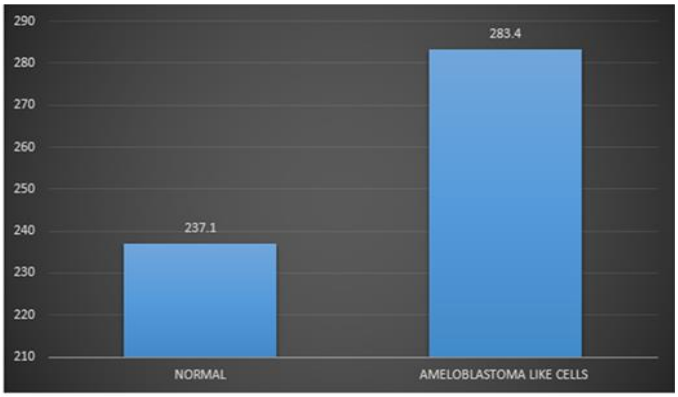
Mann Whitney u test p<0.05* significant

Table 2: comparison of positive cells among the normal tissues (ERCC1) and Ameloblastoma (Mut S)

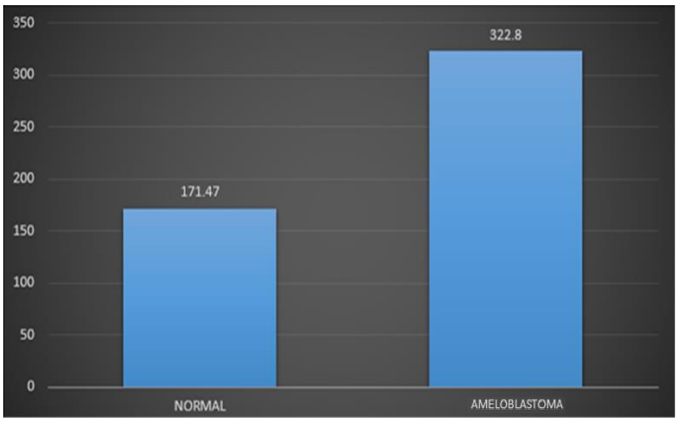
Groups	Mean	Std. Deviation	Mean ranks	Mean diff	Uvalue	Pvalue
Normal tissues	171.47	37.044	6.90	151.32	14.000	0.006*
AMELOBLASTOMA	322.8	98.72	14.1			

Mann Whitney u test p<0.05* significant

Graph 1:



Graph 2:



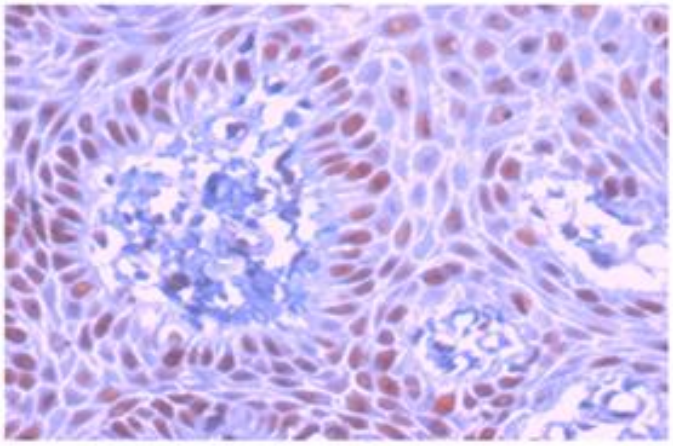


Figure 1: Showing positive MutS expression in ameloblastoma (both in ameloblastic like cells & stellate reticulum cells)

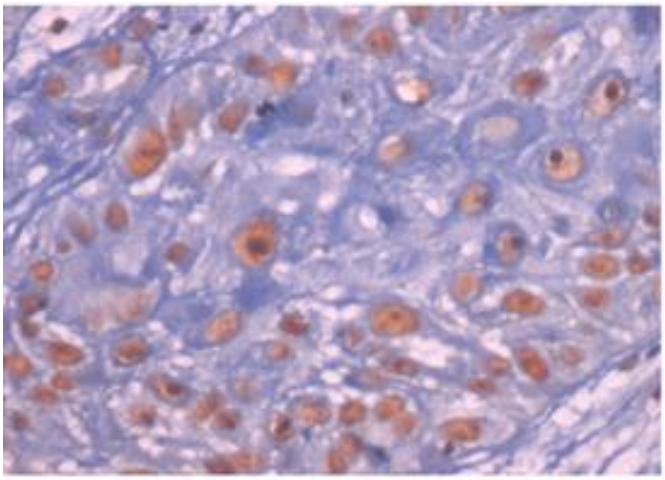


Figure 2: Showing positive MutS expression in OSCC

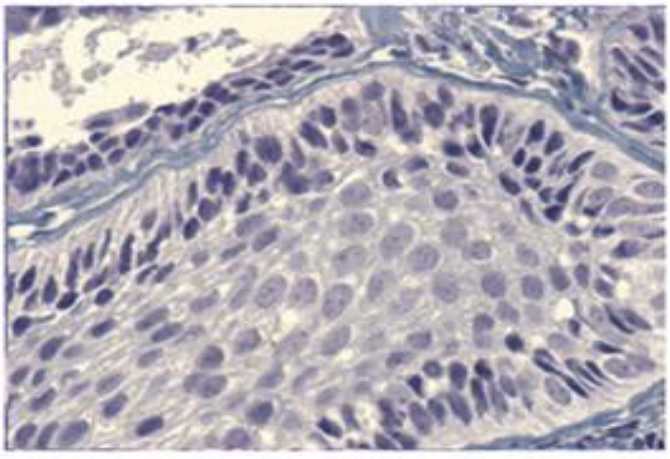


Figure 3: Showing positive expression of ERCC1 in ameloblastoma

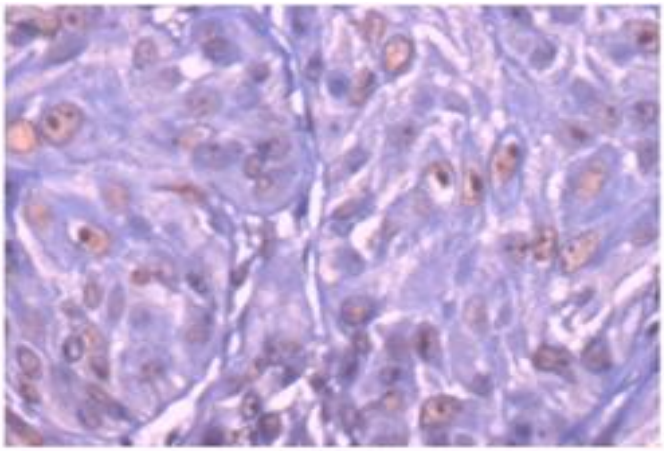


Figure 4: showing positive expression of ERCC1 in OSCC

Discussion

The oral mucosa is subjected to a range of harmful substances originating from both indigenous (such as cellular respiration) and external (such as chemical exposure) sources. Numerous unstable alterations in the cellular environment, including an increase in the generation of reactive oxygen species (ROS), changes in the cell cycle phases, the initiation of DNA damage responses, apoptosis, and many more, can result from these exposures.[2]

The cell cycle and proliferation signaling are two main biological processes that can be significantly impacted by the cells with accumulated DNA damage and defects in

repair.[2]The escape of these injured cells from cell death systems facilitates the accumulation of oncogene driver or tumor suppressor mutations specifically oriented towards malignant transformations.

Many forms of DNA damage include single-strand breaks (SSBs), double-strand breaks (DSBs), mismatch bases, oxidative base damages, abasic sites, bulky adducts, and inter- or intra-DNA cross-linkages, which can occur as a result of the cellular genome being exposed to various stressors.[2]

There exist natural and highly regulated DNA repair pathways in the human body, to outwit these damages and maintain the genomic integrity and homeostasis of the cellular environment such as base excision repair (BER) repairs, nucleotide excision repair (NER) repairs, mismatch repair (MMR) .[2]

MutS protein deficiencies lead to a mutator by interfering with methyl-directed mismatch repair, or MMR. When it comes to nucleotide excision repair, ERCC1 is crucial to the elimination of DNA intrastrand crosslinks.

Out of all the repair pathways listed above, the MMR system is an antimutagenic pathway that detects base-base nucleotide mismatches and small loops of insertion/deletion in DNA.

Histone modifications, non-codifying RNA, and DNA methylation are epigenetic mechanisms that control gene expression without changing the genomic sequence. (Rivera & Bennett, 2010). Methylation is the most common DNA epigenetic modification. Abnormal DNA methylation is associated with gene reactivation or repression and chromosomal instabilities (Gopisetty et al, 2006). DNA methylation prevents gene transcription by adding a methyl group to the cytosine of the CpG dinucleotides in the gene promoter region.

This results in transcriptional silencing of downstream genes because the presence of methyl groups promotes the remodeling of chromatin, which makes it less accessible to transcription (Khojasteh *et al*, 2013). The catalyst for this reaction known as DNA methyl transferase (DNMT) proteins DNMT3a, DNMT3b, and DNMT1 are in charge of establishing a new DNA methylation pattern and preserving the existing one throughout replication, respectively (Jurkowska et al., 2011).

In recent studies (Gomes et al., 2010; Guimaraes et al., 2015) immunohistochemical expression of DNMT was reported in ameloblastoma signifying that aberrant DNA methylation could be contributing to the etiopathogenesis of the tumor.

In this study, we attempted a comparison of MutS expression (methylation status) in normal tissues and ameloblastoma. Our results showed the higher expression of MutS in ameloblastoma when compared with normal tissues which substantiates the fact that in ameloblastoma there is a significant amount of DNA damage due to methylation. The methylation profile of MutS genes in benign lesions has not been assessed in any prior research; nonetheless, it is described in colorectal cancer has been linked to hypermethylation of these genes,

Expression of ERCC 1 is positive in the surface epithelium ameloblastic islands.

The positive expression of ERCC1 in ameloblastoma suggests that the tumour cells are experiencing a measurable degree of DNA damage that typically activates the nucleotide excision repair (NER) pathway. ERCC1 (Excision Repair Cross-Complementation Group 1) forms a functional heterodimer with XPF endonuclease, which is essential in repairing bulky DNA adducts, intra-strand crosslinks, and helix-distorting lesions through the NER mechanism. Under normal physiological conditions, the ERCC1–XPF complex

excises damaged DNA fragments, thereby maintaining genomic stability and preventing mutation accumulation.

However, despite showing positive ERCC1 immunohistochemical expression, ameloblastoma tissue often continues to accumulate DNA damage and demonstrates tumour progression. This paradox may indicate that the expressed ERCC1 protein is dysfunctional, truncated, or non-operative, potentially due to null mutations or promoter methylation affecting transcriptional efficiency. Studies on various neoplasms have shown that ERCC1 protein may be expressed yet remain non-functional due to aberrant post-translational modifications or defective complex formation with XPF. Genetic mutational analysis and promoter methylation assays are therefore required to determine whether the detected ERCC1 represents an active repair protein or a defective isoform incapable of performing excision repair.[3]

In addition, the present study demonstrated a statistically significant increase in MutS expression in ameloblastoma relative to normal mucosa. MutS protein is a critical component of the Mismatch Repair (MMR) pathway, responsible for correcting base–base mismatches and small insertion/deletion loops during DNA replication. Overexpression of MutS in ameloblastoma may indicate:

1. A compensatory upregulation in response to persistent DNA replication errors, or
2. An abnormal methylation profile, particularly hypermethylation of promoter regions, which has been reported in several tumours where MMR components are dysregulated.[4–6]

When viewed together, the positive ERCC1 and elevated MutS expression patterns suggest that ameloblastoma may rely on partially activated or dysregulated DNA repair pathways, possibly reflecting attempts of tumour cells to counteract ongoing genotoxic stress. The discrepancy between ERCC1 positivity and the tumour's inability to effectively repair DNA underscores the necessity for further molecular investigations, particularly sequencing of the ERCC1 gene, methylation-specific PCR, and studies evaluating ERCC1–XPF complex integrity. Such genetic and epigenetic data would provide deeper insights into whether ERCC1 positivity reflects:

- functional DNA repair activity or
- a biomarker of defective NER signalling leading to genomic instability and tumour progression.

Thus, the findings of this study—showing high MutS expression and positive ERCC1 immunoreactivity in ameloblastoma—highlight the significant role of DNA repair pathways in its molecular pathogenesis and reinforce the need for larger-scale studies to validate these observations.

There is a strong need for thorough research regarding MutS & ERCC1 involvement in ameloblastoma because there is subtle research focusing on ERCC1 and MutS expression in odontogenic tumors.

Conclusion

To conclude, an enhanced understanding of the molecular pathogenesis and repair mechanisms of DNA of any lesions (Ameloblastoma) might aid in developing better diagnostic techniques. Improved diagnostic techniques further help to determine the most appropriate mode of treatment for a particular patient. Only a few cases are addressed in the current study, hence an extensive examination of the involvement of ERCC1 and MutS in ameloblastoma is crucial.

Future Perspective

Understanding the interconnection between DNA repair mechanisms in perspective and ameloblastoma will aid in building up a platform for research in targeted therapies.

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