Mitochondrial antioxidants in normal oral epithelial and oral squamous cell carcinoma cell lines

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ABSTRACT

Mitochondrial antioxidants play an important role in regulation of oral cancer progression. In our study we profiled the expression levels of various mitochondrial antioxidants such as reduced glutathione, lipid peroxides, mitochondrial catalase, mitochondrial superoxide dismutase 2 (SOD2), and mitochondrial glutaredoxin 2 (GLRX2) in normal oral epithelial cell line and oral squamous cell carcinoma cell (OSCC) line. Our comparative study on these cell lines using UV-Vis Spectrophotometry and Immunoblotting reveal there is an altered profile of the mitochondrial antioxidants in oral squamous cell carcinoma. While the expression levels of mitochondrial Catalase, mitochondria Lipid Peroxide, mitochondrial Superoxide Dismutase 2 and mitochondrial Glutaredoxin 2 decrease in oral squamous cell carcinoma cell line as compared to oral epithelial cell line, the levels of reduced glutathione increase. Our results indicate there is a complex interplay of mitochondrial antioxidants in regulation of oral squamous cell carcinoma.

Figure : 01 References : 19 Tables : 02

KEY WORDS: Catalase; GLRX2; Lipid peroxide; Mitochondrial antioxidants; OSCC; Reduced glutathione; SOD2

Introduction

Oral cancer is a major public health concern worldwide, with significant morbidity and mortality rates. According to the World Health Organization, oral and pharyngeal cancer is the sixth most common cancer globally13. The incidence of oral cancer exhibits marked geographical variations, with high rates observed in certain regions such as South and Southeast Asia3,15,17. The development of oral cancer is influenced by various factors, including tobacco and alcohol use, as well as dietary deficiencies. In recent years, there has been increasing interest in the role of mitochondrial antioxidants in oral cancer. Mitochondrial antioxidants play a critical role in maintaining the balance of reactive oxygen species within cells, and their dysfunction has been linked to the pathogenesis of various diseases, including cancer4, 17, 19. In the context of oral cancer, research has shown that mitochondrial antioxidants may have a protective effect against oxidative stress, which is known to contribute to the development and progression of the disease.

Several studies have highlighted the potential impact of specific mitochondrial antioxidants, such as coenzyme Q10, on oral cancer cells. These antioxidants have been shown to modulate cellular redox status and mitochondrial function, thereby influencing the signalling...
pathways involved in cancer cell growth and survival\textsuperscript{3,17}. Understanding the mechanisms underlying the actions of mitochondrial antioxidants in oral cancer could offer new insights into potential therapeutic strategies for the disease. Research in this field has also focused on the potential use of mitochondrial antioxidants as adjuvants in conventional oral cancer treatment modalities, such as chemotherapy and radiation therapy. The synergy between mitochondrial antioxidants and traditional cancer treatment approaches hold promise for improving treatment outcomes and reducing the side effects associated with these therapies\textsuperscript{14,16}. Furthermore, the identification of specific mitochondrial antioxidant biomarkers in oral cancer patients may have diagnostic and prognostic implications, allowing for personalized treatment strategies tailored to individual patients. This personalized approach could lead to more effective and targeted therapeutic interventions, potentially improving patient outcomes and quality of life\textsuperscript{6,14,17}. As the understanding of the role of mitochondrial antioxidants in oral cancer continues to evolve, future research endeavours could explore the development of novel antioxidant-based therapies specifically tailored to target the unique metabolic and redox characteristics of oral cancer cells. Additionally, investigating the crosstalk between mitochondrial antioxidants and the tumour microenvironment may unveil novel therapeutic targets for intervention\textsuperscript{4, 9, 19}. Overall, the exploration of mitochondrial antioxidants in the context of oral cancer presents a promising avenue for advancing our understanding of the disease and developing innovative therapeutic strategies. Further research and clinical investigations in this field are warranted to fully elucidate the potential of mitochondrial antioxidants as valuable contributors to the management of oral cancer.\textsuperscript{7, 19} In our study, we compared the levels of different mitochondrial antioxidant levels such as reduced glutathione, catalase, lipid peroxide, mitochondrial superoxide dismutase (SOD2), and mitochondrial glutaredoxin 2 (GLRX2) in a normal oral epithelial cell line with oral squamous cell carcinoma cell line using UV-Vis Spectrophotometry and Immunoblotting.

### Material and Methods

The chemicals were procured from Sigma Aldrich unless otherwise mentioned.

**Maintenance of Oral Epithelial and Oral Squamous Cell Carcinoma (OSCC) Cell Line**

Oral Squamous Cell Carcinoma and Oral Epithelial Cell Lines were maintained in DMEM media supplemented with 10% Fetal Bovine Serum and 2 mM Glutamine.

**Preparation of Mitochondria from OSCC Cell Line and Normal Oral Epithelial Cell Line**

Mitochondria were isolated as described using differential centrifugation\textsuperscript{18}, and the purity of mitochondrial fraction was checked using immunoblotting against various protein markers specific to cell organelle.

**Measurement of reduced glutathione concentration in mitochondria purified from OSCC Cell Line and Normal Oral Epithelial Cell Line**

Oxidised Glutathione (GSSG) and reduced glutathione (GSH) were measured as described\textsuperscript{11} using Ellman’s method by measuring the enzymatical reaction spectrophotometrically at 412 nm.

**Catalase activity assay in mitochondria purified from OSCC Cell Line and Normal Oral Epithelial Cell Line**

Catalase activity was measured as\textsuperscript{15} by treating mitochondrial extract with Hydrogen Peroxide & 4-amino3-hydrazine-5-mercapto-1,2,24-triazole (Purpald) and measuring the enzymatical reaction spectrophotometrically at 412 nm in UV-Vis Spectrophotometer.

**Measurement of lipid peroxide content in mitochondria purified from Oral Squamous Cell Carcinoma Cell Line and Normal Oral Epithelial Cell Line**

Measurement of mitochondrial lipid peroxide content was done\textsuperscript{2,10} using thiobarbituric acid. The

### TABLE-1: Analysis of concentration/activities of reduced glutathione, lipid peroxides, and catalase in Oral Epithelial Cell Line and Oral Squamous Cell Carcinoma Cell Line

<table>
<thead>
<tr>
<th>Test molecules</th>
<th>Normal Oral Epithelial Cell Line</th>
<th>Oral Squamous Cell Carcinoma Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>$7.710 \pm 0.225$ mg/min</td>
<td>$2.8 \pm 0.321$ mg/min</td>
</tr>
<tr>
<td>Reduced Glutathione</td>
<td>$13.5 \pm 3.1$ (mM)</td>
<td>$19 \pm 2.32$</td>
</tr>
<tr>
<td>Lipid Peroxide</td>
<td>$2.65 \pm 0.167$ (nmole/mg of protein)</td>
<td>$1.23 \pm 0.745$</td>
</tr>
</tbody>
</table>
chromogen was detected spectrophotometrically at 532.5 nm. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting

The cell lysates of oral epithelial and oral cancer cell lines were fractionated by SDS-PAGE using protein gel electrophoresis apparatus (Biorad). The expression levels of SOD2 and GLRX2 in oral epithelial and oral cancer cell lines were checked using antibodies (Abcam).

**Statistical analysis**

Statistical analysis was performed done SPSS version 16.0; SPSS (Statistical Package for Social Sciences) Comparisons of biochemical parameters between Oral squamous cell carcinoma cell line and Normal Oral Epithelial Cell Line were performed using independent student t-test. The values were mentioned as mean ± SD. ANOVA test for concentration/activity levels of reduced glutathione, lipid peroxide, and catalase P values < 0.05 were considered statistically significant.

**Results**

Oral Squamous Cell Carcinoma Cell Line shows altered catalase activity, reduced glutathione level, and lipid peroxide level (Table-1). Catalase activity was reduced by one-third in the oral squamous cell carcinoma cell line. Reduced glutathione level increased and the lipid peroxide level decreased in the oral squamous cell carcinoma cell line when compared to the normal oral epithelial cell line.

The expression levels of SOD2 and GLRX 2 in normal oral epithelial and oral cancer cell lines were checked using SDS-PAGE and immunoblotting with antibodies. Expression levels of both SOD2 and GLRX2 decreased in the oral cancer cell line when compared to oral epithelial cells (Fig. 1.)

**Discussions and Conclusion**

Intracellular redox balance is maintained by reduced glutathione and in cancers reduced glutathione level is increased. In our study, we had similar findings. In a previous study of ours we found mitochondrial lipid peroxide level decrease when oral cancer progresses. A similar finding was obtained in our results on the oral cancer cell line when compared to oral epithelial cell line. Catalase prevent progression of cancer. In our earlier study, we found a decrease in mitochondrial catalase activity with the progression of cancer in patient tissue. In our study, we found decreased catalase activity in oral squamous cell carcinoma when compared to the mucosal epithelial cell line. Mitochondrial superoxide dismutase (SOD2) is a potent mitochondrial antioxidant with anti-apoptotic function. In addition, mitochondrial glutaredoxin (GLRX2) inhibits cytochrome c reductase and prevents apoptosis. In conclusion, the study of mitochondrial antioxidants in the context of oral cancer offers a promising avenue for advancing our understanding of the disease and developing innovative therapeutic strategies. The potential impact of specific mitochondrial antioxidants, such as coenzyme Q10, on modulating cellular redox status and enhancing the sensitivity of oral cancer cells to conventional cancer therapies provides a strong foundation for further research and clinical investigations. Understanding the mechanisms underlying the actions of mitochondrial antioxidants in oral cancer could offer new insights into potential therapeutic strategies for the disease. Moreover, the identification of specific mitochondrial

<table>
<thead>
<tr>
<th>Test</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean of Square</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>324.16</td>
<td>84</td>
<td>81.44</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Reduced Glutathione</td>
<td>239.34</td>
<td>76</td>
<td>83.9</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Lipid peroxide</td>
<td>185.23</td>
<td>71</td>
<td>83.81</td>
<td>0.000</td>
<td>S</td>
</tr>
</tbody>
</table>

S = statistically significant (P < 0.05), NS = statistically nonsignificant (P > 0.05).
antioxidant biomarkers in oral cancer patients may have diagnostic and prognostic implications, allowing for personalized treatment strategies tailored to individual patients. This personalized approach could lead to more effective and targeted therapeutic interventions, potentially improving patient outcomes and quality of life. Moving forward, future research endeavours could explore the development of novel antioxidant-based therapies specifically tailored to target the unique metabolic and redox characteristics of oral cancer cells. Additionally, investigating the crosstalk between mitochondrial antioxidants and the tumour microenvironment may unveil novel therapeutic targets for intervention.

In summary, the exploration of mitochondrial antioxidants in the context of oral cancer is a rapidly evolving field of study with significant implications for potential therapeutic interventions. Further research and clinical investigations in this field are warranted to fully elucidate the potential of mitochondrial antioxidants as valuable contributors to the management of oral cancer.

References


