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CASE REPORT

Case report on Reversal of Field Cancerization using Nutraceuticals mapped to the aberrant pathways deciphered via NGS analysis of the neoplastic oral mucosa

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ABSTRACT

Head and neck cancer, particularly oral cancer, presents a significant health concern in Southeast Asia with a high incidence rate, advanced stage of presentation and poor treatment outcomes. The high failure rate is due to recurrence of primary tumor and emergence of second primary tumors (SPTs) due to field cancerization. This case report focuses on a 79-year-old male with a long history of tobacco exposure, resulting in extensive field cancerization within the oral mucosa. This led to sequential development of six primaries both malignant and pre-malignant over the period of 8 years for which the patient underwent multiple surgical resections over these years. The last surgical resection specimen was subjected to Next Generation Sequencing (NGS), genetic and epigenetic alterations were identified causing dysregulation of Janus kinase/signal transducers and activators of transcription signaling pathway (JAK-STAT), Toll-like receptor signalling (TLR) and Extracellular signal regulated kinase (ERK) signalling pathways. The patient post-surgical resection is labelled as disease free; the treatment guidelines do not recommend further prescription of anticancer drugs to prevent another primary and reverse field cancerization. Also, there is lack of approved drugs targeting these pathways, a novel approach was taken using natural supplements (nutraceuticals) to modulate these dysregulated pathways. Curcumin, Epigallocatechin Gallate (EGCG) and Genistein were prescribed to downregulate TLR expression, Boswellia extracts were used to antagonize ERK activation, and Methylsulfonylmethane (MSM), Honokiol, and Berberine were employed to inhibit the JAK-STAT pathway. The patient exhibited good compliance and experienced no adverse effects. Over a span of 24 months, the patient displayed a disease-free status, and the characteristic field cancerization changes within the oral mucosa began to reverse. This unique therapeutic strategy underscores the potential of natural supplements in targeting genetic aberrations and reverting neoplastic processes, ultimately preventing the development of second primary tumors. The study highlights the significance of NGS in unravelling genomic abnormalities underlying neoplasia and supports the exploration of nutraceuticals as a viable option for correcting these aberrations. The successful application of this approach in a real-world case provides a compelling basis for further research and clinical investigation into personalized and integrative approaches for managing oral cancer and potentially other malignancies.

Introduction

In Southeast Asia, head and neck cancer is the most common malignancy, accounting for up to 50% of malignant tumours [1]. Oral cancer forms the major component of Head and neck Cancer. In India, It is estimated to be the third most common malignancy with around 77,000 new cases and 52,000 deaths are reported annually [2]. The 5-year survival rates of oral cancers in most countries are still below 50%. The high failure / recurrence after treatment of oral cancers is due to delay in the diagnosis leading to advanced stage of presentation and also emergence of the second primary tumour's (SPTs).[3] The incidence rate of second primary tumours in oral cavity is 10–35% [4].

More than 90% of oral cancers among men can be attributed to tobacco use [5]. Chewing of tobacco in form of smokeless tobacco (SLT), a monograph on SLT and Public Health in India reported that 50 per cent of oral and pharyngeal cancers can be attributed to use of SLT[6]. This chronic exposure of oral mucosa to tobacco carcinogens causes genetic and epigenetic damage in epithelial cells in one or more mucosal areas [7,8]. These changes in oral mucosa leads to development of (pre) neoplastic processes at multiple sites described as field effect or field cancerization [7,9]. Majority oral cancers develop from these precursor lesions explaining the high incidence of Second primary tumours and recurrence [10]. The altered mucosa becomes the breeding ground for newer primaries, for e.g. the patient has history of chronic tobacco abuse in form of tobacco chewing, he develops a malignancy in left buccal mucosa which is detected at an early stage and treated by surgical resection. Apart from tumor recurrence at primary site i.e. left buccal mucoa which depends on stage, grade, adequacy of treatment, etc, the patient has 10 – 35% chance of developing another malignancy at right buccal mucosa or tongue or palate due to the field cancerization caused by chewing tobacco [11]

This case report is about a 79-year-old male, who was exposed to tobacco for more than three decades and having extensive tobacco effect throughout the oral mucosa in form of field cancerization. He had undergone surgical resection 6 times for pre-malignant and malignant lesions in oral cavity. The tissue from the last resection was subjected to Next Gen Sequencing. The genetic and epigenetic alterations found were mapped to natural supplements (nutraceuticals). This led to reversal of field cancerization and delay in development of another primary.

CASE REPORT

The patient reported with a Right sided Buccal lesion in 2014 which on histopathology examination was reported as Verrucous carcinoma. The malignancy was managed by surgery in form of wide local excision with marginal mandibulectomy and neck dissection. The patient remained disease free for 8 months, thereafter he developed another lesion in the left sided buccal mucosa which was also manged by surgical resection. Subsequent years the patient developed 5 more lesions at various sites of oral cavity which were malignant as well as premalignant. The average disease-free interval between oral cavity lesions/primaries ranged between 8 to 12 months. All the surgical resections had clear margins and the neck dissection done during first two surgical procedures did not show lymph nodal metastases. The last primary was at right postero-

lateral tongue excised by us in November 2021.The histopathology reported as hyperplastic with some sites of dysplastic epithelium suggesting premalignant lesion.

We got the Next Generation Sequencing and related genomic analysis done on the excised tissue specimen.

Methods and Results from NGS analysis:

Fresh tissue from the premalignant lesion was used for the isolation of genomic DNA and RNA using Qiagen kit (Qiagen, Valencia, CA). QC qualified DNA/RNA samples were processed for NGS library preparation using Agilent DNA Prep with Enrichment kit (Cat. No. 5191-6874) and Agilent RNA Exome kit (Cat. No. 5191-6874). The QC qualified libraries were subjected to paired-end (2x150 read length configuration) sequencing on the NextSeq™ Systems (Illumina Inc., S an Diago, CA) aiming for a coverage depth of >300X. Illumina DRAGEN Somatic Pipeline (Illumina DRAGEN Bio-IT Platform v3.6) was used to analyze exome data for both DNA and RNA. The scope of analysis included SNVs, InDels, and CNAs (>6) using DNA exome data while gene fusions were detected using RNA exome data.[12]

Genomic findings and pathways altered:

A pathogenic alteration, p.Pro715Leu (rs1064794957, VCV000421171.11) in *STAT3* gene at a significant allele burden of 41% was identified, as a primary driver mutation in the complete landscape. This mutation is a gain-of-function mutation associated with activation of JAK-STAT signalling pathway. *STAT3* is altered in 1.29% of all cancers and *STAT3*p. Pro715Leu is present in 0.02% of AACR GENIE cases including oral cavity squamous cell carcinoma. The variant in not reported in population databases, 1000G and GnomAD, and is in the C-terminal Transactivation domain of *STAT3* protein [13], confirming its functional role in neogenesis. Beyond STAT3, there were somatic mutations in other pathways including the TLR4 and ERK. The *FBN1* gene had a rare mutation which was predicted pathogenic at codon 1238 (p.D1238N, RCV000181493.2). This gene is known to play an important role in ERK and TGF-β signalling pathways. We also identified a pathogenic alteration in GJB2 gene p.R32H (RCV000037873.2). GJB2 is a molecular hub and plays a central role in regulating pathways involved in oncogenesis by inhibiting apoptosis, interaction with cell adhesion molecules, promoting cell proliferation, regulating the gap junction mediated intercellular transport and communication through electric coupling, autocrine/paracrine signalling through hemichannels, positive regulation of inflammatory response through IL1 production, and TLR4 binding.

Therapy planning based on genomic findings:

It was inferred from the genomic analysis that JAK-STAT, TLR and ERK pathways were reported to be dysregulated. Since there is no approved drug in oral cancer that can be used down regulate these pathways. We did an extensive literature review and mapped certain natural supplements (nutraceuticals) which were shown to amend these pathways as shown in Table No 1.

Table No 1

S. No.	DRUG	DOSAGE	DIRECTIONS	GENES
1	CURCUMIN	2500 mg (OD)	1 sachet after lunch dissolved in 200ml water	TLR
2	EGCG	500 mg (OD)	1 capsule 30 min before lunch with half a glass of warm water	TLR
3	MSM	1000 mg (BD)	1 capsule 2 hour after breakfast and 1 capsule 2 hour after lunch	JAK INHIBITOR
4	HONOKIOL	250mg (BD)	1 capsule with breakfast and 1 capsule with dinner	STAT3
5	BERBERINE	500mg (BD)	2 capsules with lunch and 2 capsules with dinner	JAK INHIBITOR
6	AKBA	200mg (BD)	1 capsule Morning and 1 capsule before sleep	ERK
7	GENISTEIN	125mg (TID)	1 capsule with breakfast, 1 capsule with lunch and 1 capsule with Dinner	TLR

The patient was prescribed the mentioned nutraceuticals in the recommended dosages with due regulatory approval. The patient has shown good compliance and no adverse effects were noted. The last follow up was done in Septmeber 2023, patient had not developed any new lesion Also, on clinical examination the field cancerization changes in form of discoloured patches in oral mucosa has started to revert.

Discussion

We have tried to review the effect of each of the above-mentioned genetic aberrations in carcinogenesis of oral cancer and how the prescribed nutraceuticals correct the aberrations.

TLRs (Toll-like receptors) play a complex role in cancer development and progression. When stimulated, TLRs have tumor-promoting effects by activating pro-inflammatory signalling pathways, leading to the release of pro-inflammatory cytokines and chemokines [14]. This creates an inflammatory environment that supports tumor growth, angiogenesis and metastasis [15]. Additionally, TLR activation can suppress anti-tumor immune responses, enabling tumors to evade immune surveillance [16].

ERK activation occurs in response to extracellular signals, such as growth factors and hormones [17]. This activation involves a series of phosphorylation events, ultimately leading to the activation of ERK1 and ERK2 [18]. Once activated, ERK1/2 translocate to the nucleus, where they phosphorylate various target proteins, regulating gene expression and cellular processes [18]. Dysregulation of the ERK pathway contributes to tumor development and progression in form of uncontrolled cell proliferation, inhibits apoptosis, enhances tumor cell invasion, and stimulates angiogenesis [18].

The JAK-STAT pathway is activated by cytokines or growth factors binding to their receptors, leading to the activation of Janus kinases (JAKs) [19]. JAKs phosphorylate tyrosine residues on the receptor, providing docking sites for cytoplasmic STAT proteins. Phosphorylated STAT proteins form dimers, translocate to the nucleus, and act as

transcription factors, regulating the expression of target genes involved in cell growth, survival, differentiation, and immune responses [20]. Dysregulation of the JAK-STAT pathway in cancer can promote abnormal cell proliferation, inhibit apoptosis, contribute to chronic inflammation, modulate immune responses, and promote angiogenesis and metastasis [21]. Additionally, JAK-STAT signalling can facilitate immune evasion by cancer cells and contribute to angiogenesis and metastasis [22].

In summary, TLRs, the ERK pathway, and the JAK-STAT pathway are all implicated in cancer development and progression. TLR activation promotes tumor growth, suppress anti-tumor immune responses, and inhibit apoptosis. Dysregulation of the ERK pathway leads to uncontrolled cell proliferation, tumor growth, invasion, and resistance to therapy. Abnormal activation of the JAK-STAT pathway drives cell proliferation, inhibits apoptosis, contributes to inflammation and immune evasion, and promotes angiogenesis and metastasis. In order to, amend the aberration in above mentioned pathways; certain natural supplements (nutraceuticals) were mapped against the pathways.

Curcumin, EGCG and Genistein were prescribed to downregulate TLR expression. Curcumin and EGCG inhibit the binding of various ligands generated by changes in oral mucosa caused by tobacco abuse to TLR, such as lipopolysaccharides (LPS) and DNA fragments like CpG (*5′—C—phosphate—G—3′*, that is, cytosine and guanine separated by one phosphate group)DNA[23, 24]. They also inhibit downstream signalling pathways of TLR such as transcription factor nuclear factor-kappa B (NF-κB)[25] and other pathways such as the MAPK (Mitogen-activated protein kinase) EGCG has also been shown to induce the expression of negative regulators of TLR signalling, such as the protein suppressor of cytokine signalling 1 (SOCS1), which can further inhibit TLR signaling.[26,27] Genistein also inhibits TLR on similar lines as Curcumin and EGCG. Additionally, it has been shown to downregulate the expression of TLR4, as well as the expression of the downstream signalling molecules MYD88 and TRAF6 [28]

Boswellia extracts were considered to antagonise ERK expression. They have been shown to inhibit the activity of enzymes called protein tyrosine kinases, which are involved in the activation of the ERK pathway.[29] They also reduce the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), which can activate the ERK pathway.[30] Some studies depict Boswellia extracts to directly bind and inhibit the activity of the kinase itself.[31]

MSM, Honokiol and Berberine were considered to down regulate JAK-STAT pathway. MSM and Berberine inhibit the activity of protein tyrosine kinases[32,33] as well as reduce the production of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), both of which are known to activate the JAK-STAT pathway.[34,35,36] It also inhibits the production of reactive oxygen species (ROS), which can activate JAK and contribute to the development of inflammation.[37] Berberine also inhibits the phosphorylation and activation of STAT3, which is downstream of JAK [38]. Honokiol has been shown to bind to the SH2 domain of STAT3, which prevents its phosphorylation and subsequent activation.[39]

The patient was prescribed the above mentioned in standard recommended doses.

The patient is on regular follow up which is now more than 24 months. There is no incidence of another primary and the field cancerization changes have also started to revert.

Conclusion

Chronic tobacco abuse leads to development of premalignant changes across all the exposed oral mucosa also termed as field cancerization which is a major reason for development of Second Primary in oral cancers. With advent of NGS, it is now possible to understand the genomic aberrations leading to formation of neoplasia. Until, we have standard of care drugs to correct the genomic aberrations, we recommend to explore the option of using natural supplements (nutraceuticals). It might help revert neoplastic process as in the present case, we were able to revert field cancerization and prevent another primary tumor.

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