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Research Article

IMMUNO HIS TO CHEMICAL EXPRESSION OF SMAD-2: A MESENCHYMAL STEM CELL MARKER IN OSSIFYING FIBROMA

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A B S T R A C T

Ossifying fibroma (OF) is a common benign fibro-osseous neoplasm of the jaws with progressive enlargement of the affected jaw causing significant cosmetic and functional disturbances. Complete surgical removal with safe margin is the recommended treatment for OF. It has a high recurrence rate. An understanding of the molecular mechanisms behind them will be more beneficial for application of targeted therapies in such lesions.
A plethora of stem-cells have been identified in a vast array of tumors, especially in malignancies for applying targeted therapy. But their role in benign tumors is not yet determined. Studies have shown that OF show more proliferative nature of stromal-cells. The aggressive growth may be attributed to mesenchymal-stem-cell population which mediates through enhanced TGF- β signaling in a SMAD-2/3 dependent manner. This contributes to OF phenotype characterized by suppressed bone formation and aggressive stromal tissue growth. Hence the study was planned to evaluate immune his to chemical expression of SMAD-2 in OF.
10 Paraffin embedded tissue sections of his to pathologically diagnosed cases of OF were immune his to chemically stained with SMAD-2 & its nuclear expression was evaluated.
Descriptive statistical analysis was applied. The significant positive expression of SMAD-2 in present study revealed the role of mesenchymal-stem-cells mediating through TGF-β signaling in OF.

INTRODUCTION

Ossifying fibromas (OF) of the craniofacial skeleton are benign fibro-osseous neoplasms characterized by the replacement of normal bone by a fibrous cellular stroma containing foci of mineralized bone trabeculae and cementum-like material that vary in amount and appearance. They have formed an intriguing aspect of a variety of fibroosseous lesions, probably owing to their wide histo-morphologic spectrum. Menzel in 1872 first described this entity and Montogmery coined the term "Ossifying fibroma" in 1927.[1]
Currently, complete surgical removal of the affected bone with safe margin is widely recommended treatment for OF. However, patients often suffer from difficult reconstruction with postsurgical disfigurement and major loss of vital tissues.[2] It has a high recurrence rate. The indisputable acceptance of safe margin surgical resection of this benign tumor as standard of care reflects current knowledge gap in the pathophysiology of OF and should be reexamined based on current understanding of tumor cell biology. A cornucopia of tumor stem cells have been identified in a broad spectrum of tumors, especially in malignancies. [3] This population of cancer stem cells usually accounts for a small percentage of bulk tumor cells but is regarded as a driver of tumor growth, progress, metastasis & recurrence, implying that effective therapy should be targeted at this population of cells [4]. However the detailed molecular mechanisms & regulatory networks that determine stem cell function in most benign tumors including OF, are unknown.

OF is believed to arise from the undifferentiated mesenchymal cells of the periodontal membrane which serve as multipotential precursor cells capable of differentiating into cementum, osteoid, or fibrous tissue and give rise to a spectrum of fibro-osseous lesions.[4]

Studies have shown that OF show more proliferative nature of stromal cells. The aggressive growth may be attributed to mesenchymal stem cell population which mediates through enhanced TGF beta signaling in a SMAD 2/3 dependent manner.[5] This contributes to OF phenotype characterized by suppressed bone formation & aggressive stromal tissue growth. Hence the study was planned to evaluate the immunohistochemical expression of SMAD 2 in OF.

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MATERIALS & METHODS

10 his to pathologically diagnosed cases of ossifying fibroma were taken as a study group. 3-µm sections were cut from the paraffin embedded tissue blocks & stained immune his to chemically with rabbit polyclonal antibody SMAD2 from Abcam & its nulear expression was evaluated. Breast carcinoma tissues were taken as positive control whereas normal bone tissue obtained during surgical removal of third molars were taken as negative control. Five higher magnification fields were examined for SMAD 2 expression (Fig 1 and 2).

The present study was conducted in the Department of Oral Pathology and Microbiology, Government Dental College & Hospital, Aurangabad in collaboration with Department of General Pathology, Government Medical College and Hospital, Aurangabad with the approval of Institutional Ethical Committee during the period of 2015-16.

RESULTS

The mean percentage of cells showing positive SMAD 2 expression was calculated (using Table 1) and descriptive statistical analysis was applied. Mean positive expression of SMAD-2 for each case in five higher magnification fields is shown in the Table 2. Results revealed 72.78% positivity (mean) for expression of SMAD 2 with SD of 3.062 indicating the role of mesenchymal stem cells mediating through TGF beta signaling in ossifying fibroma.

Table 1 Results for SMAD- positive expression in five higher magnification fields in each case

	Field 1	Field 2	Field 3	Field 4	Field 5
Case 1	67	87	75	62	80
Case 2	68	69	83	72	81
Case 3	75	61	64	70	68
Case 4	85	68	63	74	76
Case 5	73	84	80	76	68
Case 6	79	83	86	74	80
Case 7	63	60	58	67	62
Case 8	60	59	57	52	51
Case 9	88	82	89	87	87
Case 10	74	68	79	85	80

Table 2 Results for mean SMAD-2 Positive expression for each case

Case No.	Mean SMAD 2 Positive Expression
Case 1	74.2
Case 2	74.6
Case 3	67.6
Case 4	73.2
Case 4	76.2
Case 5	80.04
Case 6	62
Case 7	55.8
Case 8	86.6
Case 9	86.6
Case 10	72.2

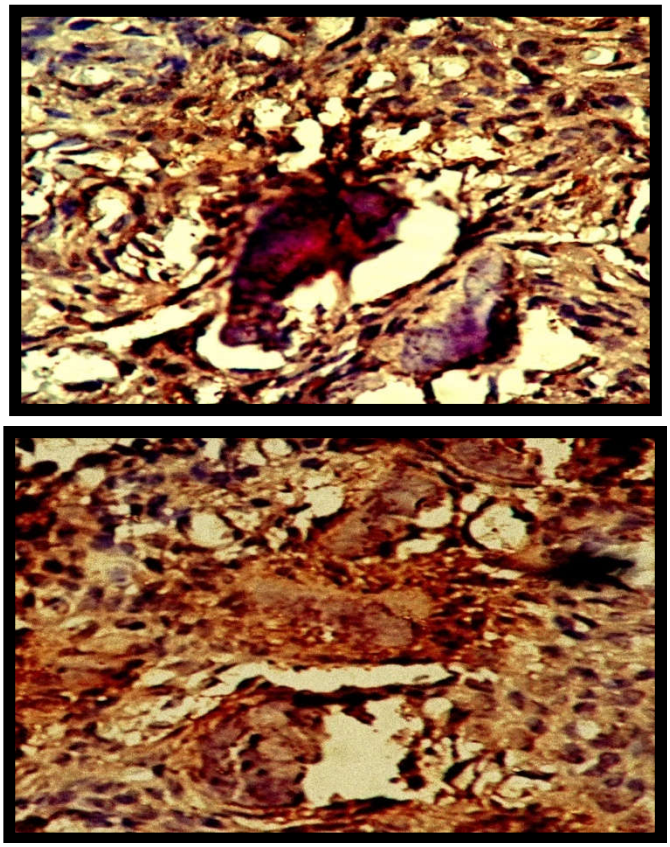


Fig. 1 & Fig 2 Nuclear Expression of SMAD-2 in OF

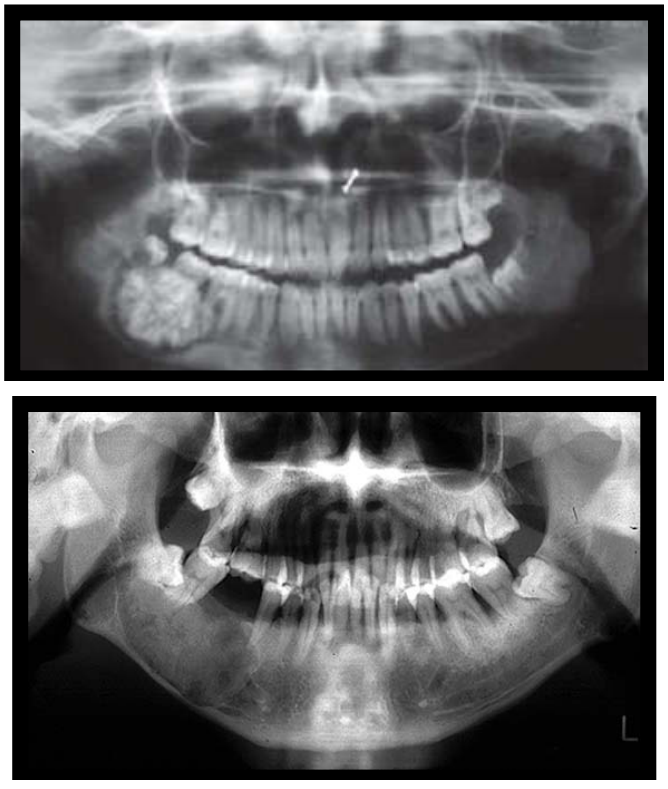
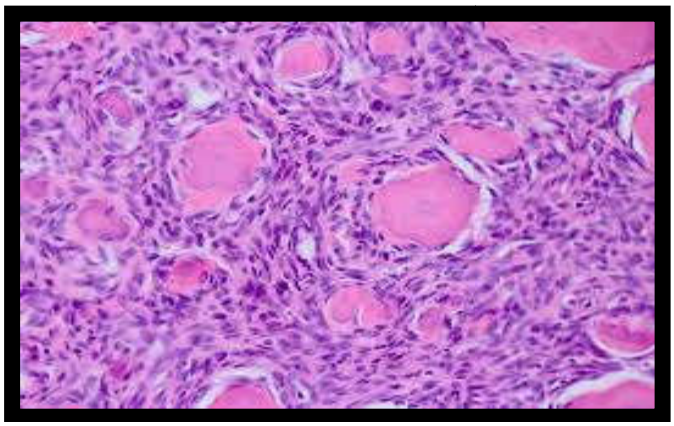


Fig. 3 & 4 Radiological Variations in OF



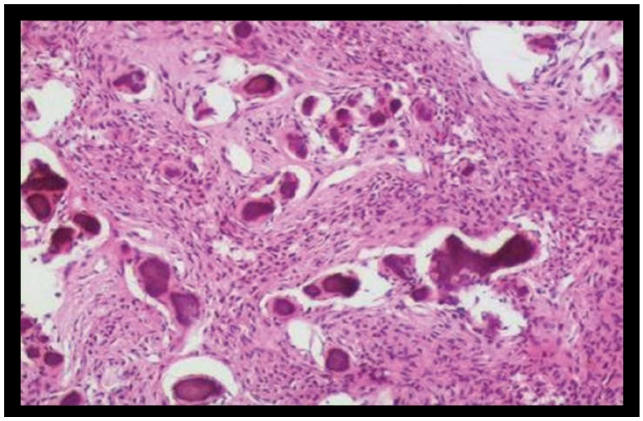


Fig 5 & 6 Histological Variations in OF

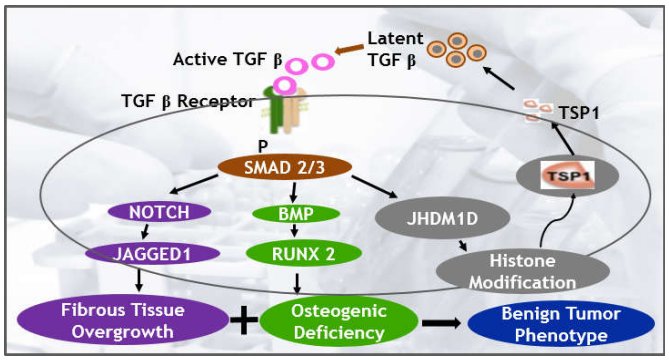


Fig 7 Signaling Mechanisms in OF

DISCUSSION

"Ossifying fibroma (OF) is a well-demarcated lesion composed of fibrocellular tissue and mineralized material of varying appearances" (WHO 2005). Conventional & juvenile are the two variants of ossifying fibroma amongst which juvenile is further subclassified as trabecular and psammomatoid variants. Among the 3 subtypes, conventional OF is the most common and usually affects female adults in their 2nd to 4th decades of life. More than 70% of conventional OF cases are reported to affect the mandible, particularly in the posterior regions. Painless bone swelling is the most common sign associated with this condition. It can induce swelling of the buccolingual and inferior mandibular cortical bone in $\leq$ 30% of cases, displacement of the inferior mandibular canal in > 50% of cases, involvement of the maxillary sinus in > 80% of maxillary cases, displacement of teeth/ roots in $\leq$ 25% of cases, and root resorption in $\leq$ 20% of cases.[6]

Radiographically radiodense as well as radiolucent areas may be seen depending upon the hard and soft tissue components (Fig. 3 and 4). Histologically it may exhibit the fibrous tissue that varies in cellularity and is usually distributed in a storiform pattern with interspersed trabeculae of woven and lamellar bone and/or spherules of cementum-like material (Fig. 5 and 6).[6]

Amongst the subtypes of OF, Juvenile psammomatoid OF is found to be having more aggressive phenotype.[7] As previously introduced, because of the aggressive behavior & surgery as the only treatment option available the quality of life is largely compromised and there is an urgent need for understanding of the molecular mechanisms & to develop target specific treatment in OF.

Mesenchymal stem cells (MSCs) are stromal progenitor cells capable of self-renewal, multilineage differentiation, and immunomodulation. MSCs have therefore been used in clinical settings for tissue regeneration and immune therapies. Additionally, multiple lines of evidence indicate that stem cell properties of MSCs may affect cancer and benign tumor behavior. Xu *et al* (2009) revealed cancer stem cells especially in malignancies.[3] Visvader and Lindeman (2012) evaluated the role of cancer stem cells in tumor growth, metastasis & recurrences which may be useful for targeted therapies.[8]

Among the different signaling pathways involved in MSC proliferation and differentiation, TGF- β signaling is of interest because it has been reported to be associated with both stem cell function and tumor development. Massague (2008) evaluated the role of TGF beta signaling in stem cell function & tumor development.[5]

Jian *et al* (2006) shown the role of TGF beta in mesenchymal stem cell proliferation. TGF-β signaling enhances MSC proliferation via nuclear translocation of β -catenin in a SMAD 2/3-dependent manner and inhibits MSC differentiation via repression of RUNX2 function.[9] There are less studies on involvement of TGF-β signaling in the development of mesenchymal cell-associated benign tumors. The Study by Qin H *et al* revealed that OF tumors contain mesenchymal stem cells (OFMSCs) capable of recapitulating the parental tumor phenotype when implanted in vivo.[10]

These OFMSCs are capable of forming single-colony clusters, expressing specific stem cell surface markers, responding to differentiating induction cues, and generating tumors when implanted in vivo, thus meeting the primary criteria for classification as cancer stem cells.

Basically TGF-β enhances MSC proliferation via nuclear translocation of β-catenin in a SMAD 2/3-dependent manner. There will be phosphorylation of SMAD & downstream expression of genes.[10]

In OF, TSP1-activated TGF-β promotes fibro-osseous stromal cell growth and induces osteogenic deficiency. Intriguingly, TSP1 expression in OF is under the regulation of TGF-β signaling, forming a positive feedback loop that strengthens the activity of TGF-β signaling. This TGF β-driven TSP1 expression is regulated by the JHDM1D-associated histone modification in OF MSCs.[10]

TGF B signaling in OF is mediated through SMAD 2/3. TGF-β is secreted into the extracellular matrix in a latent form which is subsequently cleaved into an active form to serve its

regulatory function. Active TGF-β is found to be significantly increased in OFMSCs. This is responsible for increased phosphorylation of SMAD 2 & 3. It will in hibit the BMP signaling & will cause repression of RUNX2 which will ultimately inhibit osteogenic differentiation leading to bone loss.[10]

Increased phosphorylation of SMADs is also responsible for upregulating Notch signaling which will cause elevation of expression of specific notch signaling genes like Jagged1 in OFMSCs which will contribute to elevated cell proliferation & stromal tissue growth.[10]

TSP1 is known to facilitate the conversion of latent TGF-β to active TGF-β & TSP1 expression is also found to be elevated in OFMSCs contributing to the activation of TGF-β signaling in OFMSCs. Thus the autocrine loop is found to be continuously responsible for pathophysiology in OF (Fig.7).[10]

Knockdown of TGF-β signaling in in-vivo studies revealed elevated osteogenic gene expression and in vivo bone formation i.e. enhanced osteogenesis of OFMSCs.[10]
Thus blockage of TGF β signaling and its autocrine components in OFMSCs may provide a unique therapy for OF lesions.

Significant positive expression of SMAD2 in our study supported the role of TGF β signaling in the pathogenesis of OF through the formation of OFMSC phenotype. Thus targeting any one of the molecule of TGF-β signaling may restrict the aggressive behavior of OF preventing the required wide surgical resection. Still further studies using large samples are still needed to evaluate the role of TGF-B signaling in OF.

To conclude, SMAD-2 can be used as a mesenchymal stem cell marker in ossifying fibroma and with an increased understanding of the mechanism of OFMSC, it could be possible to stop the harmful growth of OF before risky surgery is needed.

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Conflicts of Interest: None

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