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TGF-β PARADOX AND THE PROGRESSION OF OSTEOBLASTOMA VERSUS OSTEOSARCOMA

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ABSTRACT

Objective: To study the expression of Transforming Growth Factor beta-1 (TGF-β1) and Tumor protein 53 (Tp53) in osteoblastoma and osteosarcoma and the role of TGF-β paradox in the progression of these tumors.

Materials and Methods: Twenty-two paraffin blocks were collected and classified into 7 blocks with classical osteoblastoma and 15 blocks with osteosarcoma. From each block, slide preparation for H&E and immunohistochemical staining by two markers: anti TGF-β1 antibody and anti Tp53 antibody were applied. Then we estimated the expression of each marker by ImageJ (version 1.41) and area fractions were measured.

Results: Both classical osteoblastoma and osteosarcoma showed positive expression of TGF-β1. The expression of Tp53 was positive in osteosarcoma, but it was negative in osteoblastoma. Our statistical analysis revealed that the expression of TGF-β1 in osteosarcoma was stronger than in osteoblastoma but with no statistically significant difference. There was a significant moderate negative correlation between expression of the two markers in osteosarcoma.

Conclusion: The mutual signaling is the main pillar in the progression of osteosarcoma and osteoblastoma. Interaction pathways between Tp53 and TGF-β1 may have a role in TGF-β paradox. Understanding this interaction may help in improving of the management and prognosis of osteosarcoma.

KEY WORDS: osteoblastoma; osteosarcoma; TGF-β1; Tp53 and TGF-β Paradox

INTRODUCTION

A classical osteoblastoma is a rare benign tumor of jaw bone with no opportunity to give a malignant transformation (Mohseny *et al*., 2012). It is characterized histologically by the presence of normal osteoblasts, osteoid and immature mineralized tissue (Oliveira *et al*., 2007). Osteosarcoma of the jaw bone is a high-grade rare neoplasm. (Bertin, Gomez-Brouchet, & R´edini, 2020).

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The stroma is composed of extracellular matrix (ECM) and cells. A neoplastic stroma has a major role in cancer growth and progression (Verrecchia and Rédini, 2018). There are two types of mutual signaling: the first one is between tumor cell and ECM (Sainioa and Järveläinena, 2020). The ECM - cell interaction is reciprocal, neoplastic lesions develop as a result of the disturbance in ECM - cell signaling mechanisms (Sanderson *et al*., 2017).

The second mutual signaling occurs between tumor cells and corresponding non-neoplastic cells in the surrounding micro-environment. The mutual signaling between osteosarcoma cells and their corresponding normal non-neoplastic osteoblasts and osteoclasts is considered as a main reason for pathogenesis and progression of this cancer (Verrecchia, and Rédini, 2018). This signaling controls the secretion of many cytokines and extracellular matrix elements like transforming growth factor beta (TGF-β) (Verrecchia and Rédini, 2018).

In normal bone, there is a balance between bone resorption and formation. This balance is guided by different factors. TGF-β1 is one of these factors which is considered as a growth factor that controls the differentiation of osteoblasts and osteoclasts (Kasagi, and Chen, 2013; Lamora et al., 2016). Also, TGF-β1 controls the interaction between osteoclasts and osteoblasts. TGF-β1 is released from the bone matrix during bone resorption and is responsible for the migration of mesenchymal stem cells and their differentiation into osteoblasts at the resorption site (Kim et al., 2020). **Janssens et al**, **2005** reported that high concentration of TGF- β1 leads to inhibition of osteoclast differentiation and enhancement of osteoblastogenesis.

TGF-βs have a dual effect as tumor suppressor or promotor according to the tumor type and the phase of tumor development (Lamora et al., 2016). According to tumor type, TGF-βs works as tumor suppressor in the benign tumors and as tumor promotor in the malignant tumors which is called TGF-β paradox (Zhang, Yu, & Lee, 2014). In the early phase of tumor development, TGF-β1 acts as a tumor suppressor, but in the late stage of tumor development, TGF-β1 acts as tumor promotor through the induction of epithelial-mesenchymal transition, angiogenesis and immunosuppression (Tang et al., 2018).

The normal Tp53 is "the Guardian of the Genome", guarding against genetic instability of replicating cells thus preventing malignant transformation (Vousden and Carol, 2009). Once activated, Tp53 promotes transcriptional activation of apoptotic genes (Nakano and Vousden, 2001) and tumor suppression genes (including TGF-β1) (Wilkinson, Ogden, and Stratton, 2005). On the other hand, mutated Tp53 plays a role in tumor progression through induction of chromosomal instability, disruption of cell cycle control, apoptosis and DNA repair mechanisms (Goh, Coffill, and Lane, 2011).

The interaction between the Tp53 pathway and the TGF-β1 pathway is still unclear and if this interaction plays a role in the progression of benign and malignant tumors of bone. This interaction appears in regulation of cell cycle progression, differentiation and tumor suppression (Wilkinson, Ogden, and Stratton, 2005). Understanding the interaction between ECM TGF-β1 and Tp53 will help us know the progression of certain tumors, helping in the diagnosis, treatment and understanding the prognosis of the lesions.

Normal Tp53 induces a tumor suppression effect of TGF-β1 via p21 (Adorno *et al*., 2009). But, mutated Tp53 induces a tumor promotion effect of TGF-β1 (Elston, and Inman, 2012) facilitating tumor cell proliferation, angiogenesis and suppression of immune system (Lamora et al., 2016; Tang *et al*., 2018)

In the current study, we will investigate the expression of TGF-β1 in classical osteoblastoma

and osteosarcoma and the role of TGF-β paradox in the progression of two types of bone tumors, through studying the interaction between extracellular matrix TGF-β1 and Tp53.

MATERIALS AND METHODS

Blocks:

Twenty-two paraffin blocks were collected at Minia University Dental Hospital and National Cancer Institute - Cairo University. They were fifteen cases of osteosarcoma and seven cases of osteoblastoma.

Hematoxylin and Eosin (H & E) staining:

Using H & E stain, re-evaluation of these cases was carried out to confirm the diagnosis. From each block, slide preparation was performed. The fixed slides were rehydrated in descending concentrations of alcohol, washed in distilled water for 5 minutes. The slides were immersed in filtered hematoxylin stain for 3 minutes and then washed with distilled water twice. The slides were immersed in filtered eosin stain for 5 seconds and then washed with distilled water. Dried slides were immersed in xylene, mounted with Canada balsam then cover slips were placed and left to dry (Llewellyn, 2009).

Immunohistochemical Staining:

For all specimens, 4-microns thick paraffin embedded tissue sections were prepared and mounted on positively charged glass slides. The sections were deparaffinized by warm xylene, rehydrated in descending concentrations of alcohol and immersed in phosphate buffered-saline (PBS). The slides were completely immersed in a path of antigen retrieval solution (pH 9). Then slides were incubated by PBS and treated by 0.3% H_2O_2. The primary Rabbit polyclonal anti Tp53 Antibody - Abcam-ab131442 – (1:100) was applied to cover the sections completely followed by incubation at room temperature for overnight. Then the slides were washed by PBS. The slides were completely covered with secondary antibody HRP Envision kit (DAKO) for 20 mins; the slides were washed by PBS and incubated with diaminobenzidine (DAB) for 10 mins. Washed by PBS then counter staining with Hematoxylin Harris, dehydrated of the section in ascending concentration of alcohol and cleared in xylene. Then cover slipped for microscopic examination and quantification. All these steps were repeated with the primary anti TGF-β1 antibody - NBP2-22114– Novus inc. (1:100) (Kabiraj *et al.*, 2015).

Photomicrography analysis:

H & E slides were photographed using a digital video camera LEICA DFC295 which was mounted on a light microscope in histopathological laboratory in Minia University Dental Hospital. Then images were transferred to the computer system for analysis.

Morphometric analysis:

All the steps performed for immunohistochemical evaluation were carried out using image analysis software (ImageJ, version 1.41). Phase analysis was calculated automatically to give the percentage of immunopositivity area to the total area of microscopic field. Image analysis was performed at the Oral Pathology Department, Minia University Dental Hospital.

Statistical analysis:

The collected data were tabulated using Microsoft Excel (Microsoft Office 2019). The mean area fraction for each case was then calculated and used for statistical analysis. The data was stored and analyzed by SPSS 20 for windows. For immunostaining data, paired sample t-test, independent sample t-test, and correlation tests were used for continuous parametric data. Significance level was set as ≤ 0.05.

RESULTS

H &E Findings

H & E staining slides showed that the seven cases of osteoblastoma were a classical type with inter-anastomosing of normal trabeculae of woven bone rimmed by a single row of normal osteoblasts layer (Figure 1 a.). Fifteen cases of osteosarcoma showed the proliferation of atypical osteoblastic cells and the production of osteoid tissue and immature bone (Figure 1 b.).

Immunohistochemistry Findings

In classical osteoblastoma, the expression of Tp53 was negative in the extracellular stoma, osteoblastic rimming layer and mineralized tissue in as shown in (Figure 2). The TGF-β1 expression was detected in extracellular stoma of osteoblastoma: extracellular matrix and cells, and in osteoblastic rimming layer but the mineralized woven bone tissue was negative for TGF-β1 (Figure 3).

Regarding to osteosarcoma, the expression of Tp53 was detected in nucleus and cytoplasm of osteosarcoma cells but Tp53 had a negative expression in immature woven bone tissue (Figure 4). The TGF-β1 expression was detected in extracellular stroma: extracellular matrix and cells, endothelial cells, perivascular cells and osteoclasts. Also, TGF-β1 was detected in (unmineralized bone tissue) osteoid tissue but it is absent in mineralized immature bone tissue (Figure 5).

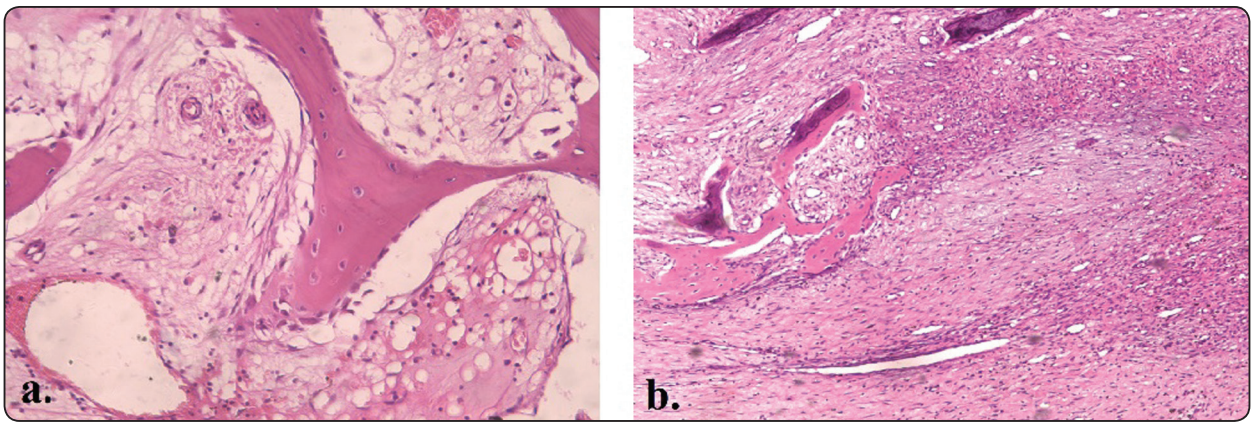

Fig. (1). (a & b): a. Histologic view of osteoblastoma showing of woven bone rimmed by a single row of osteoblasts (x20). b. Histologic view of osteosarcoma showing atypical osteoid tissue formation in vascularized sarcomatous stroma (x10).

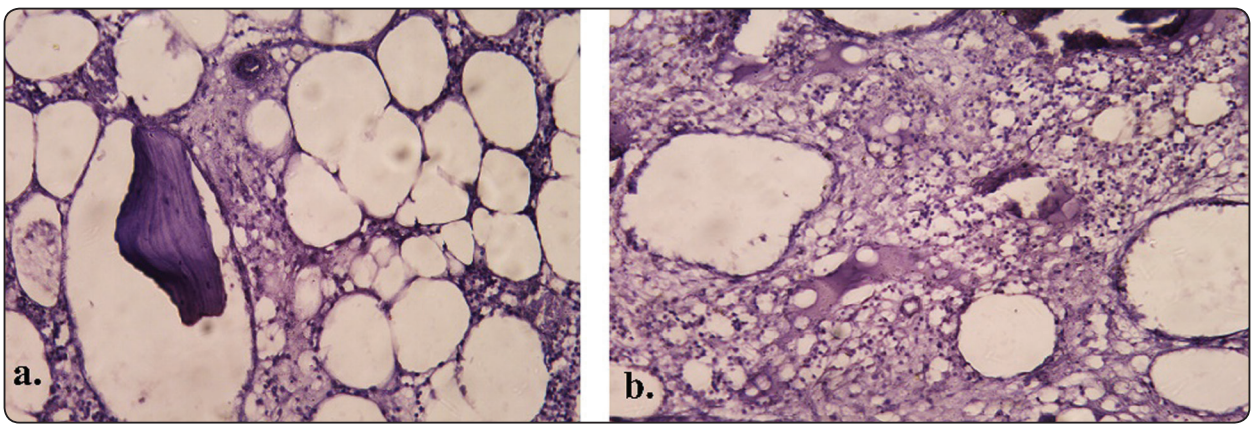

Fig. (2). a & b): Histologic view of osteoblastoma showing immune-negative reactivity of Tp53 (x20).

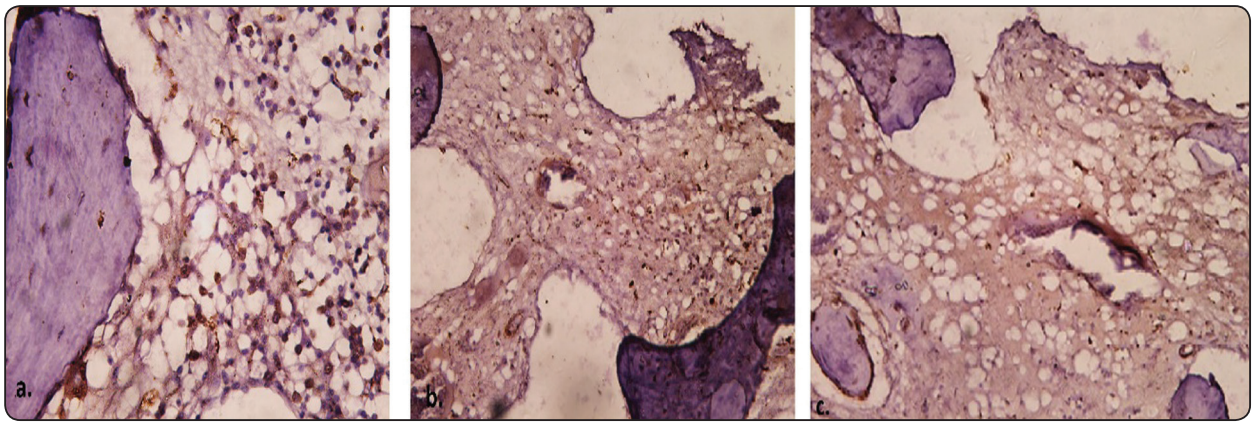

Fig. (3). a, b & c): Histologic view of osteoblastoma showing: a. immune-positive reactivity of TGF-β1 in nucleus (x40), b. immune-positive reactivity of TGF-β1 in extracellular stroma (x20) and c. immune-positive reactivity of TGF-β1 in osteoblastic rimming and extracellular stroma with immune-negative reactivity of TGF-β1 in bone tissue (x20).

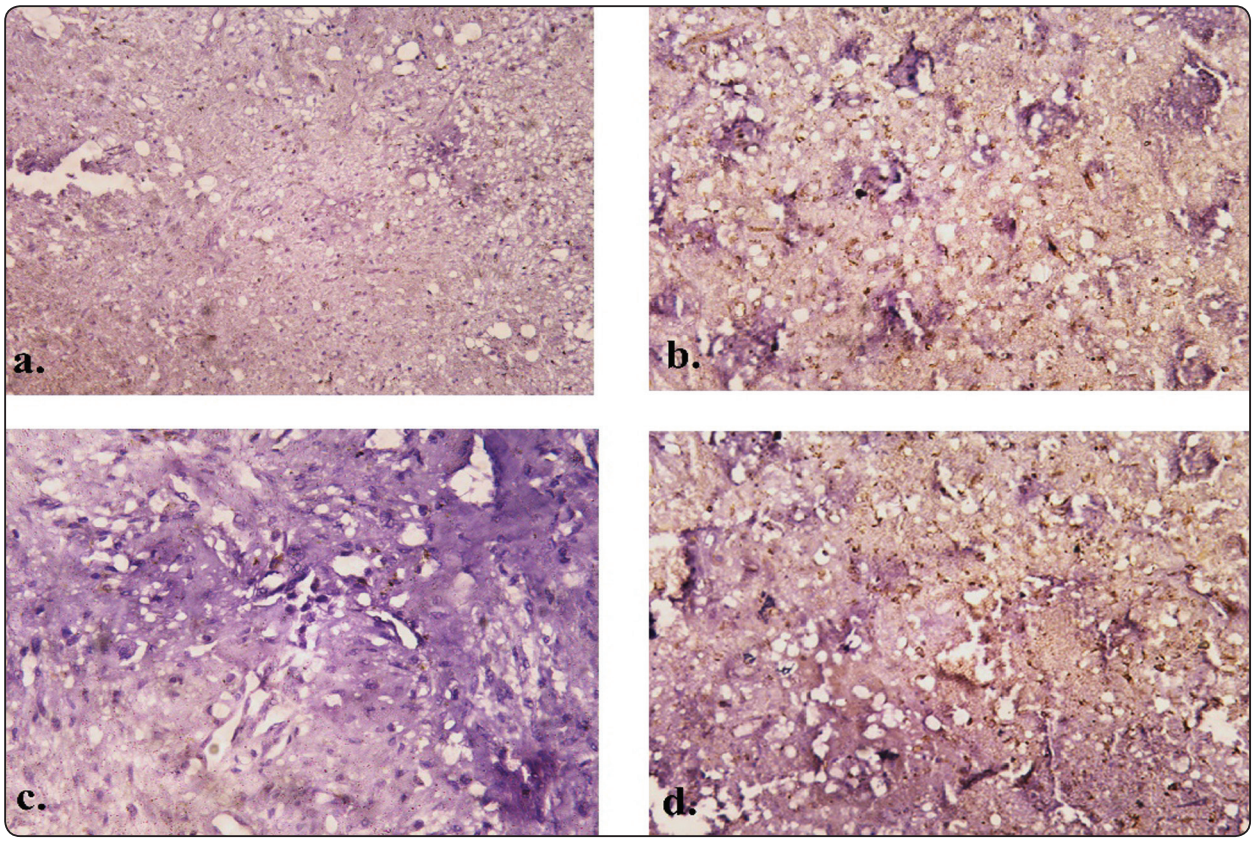

Fig. (4). a, b, c & d): Histologic view of osteosarcoma showing: a & b. immune-positive reactivity of Tp53 nucleus and cytoplasm of cells (a.x10 & b.x20) and c & d. immune-negative reactivity of Tp53 osteoid tissue (c.x40 & d.x20).

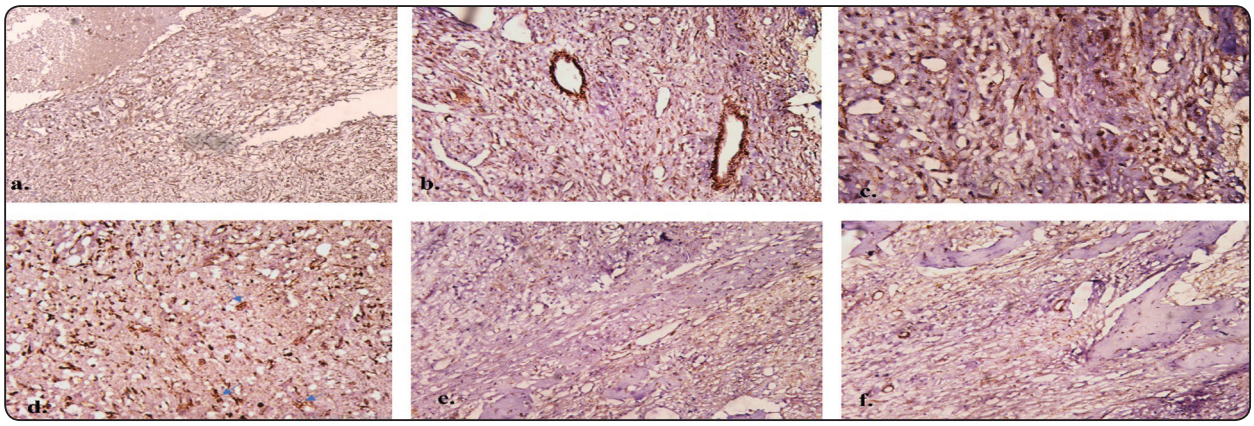

Fig. (5). a, b, c, d, e &f): Histologic view of osteosarcoma showing: a. immune-positive reactivity of TGF-β1 in nucleus and extracellular stroma (x10), b & c. immune-positive reactivity of TGF-β1 in endothelial and perivascular layers and osteoid tissue (b.x20 & c.x40), d. immune-positive reactivity of TGF-β1 in osteoclast cells (blue arrow) (x20), and e & f. immune-negative reactivity of TGF-β1 in mineralized bone tissue (x20).

Statistical Analysis

Results of immunohistochemical expression of TGF-β1 in osteoblastoma and osteosarcoma were compared using the independent samples t-test and there was an insignificant difference between the expression of TGF-β1 in the two tumors. (p= 0.375)

Results of immunohistochemical expression of Tp53 and TGF-β1 in osteosarcoma were compared using paired samples t-test and there was highly significant difference between the two markers (p= 0.004). Then, correlation test was carried out between the two markers in osteosarcoma and there was a medium negative correlation between them (r= -.553, p= 0.041)

DISCUSSION

The progression of tumors depends mostly on the mutual signaling between neoplastic cells and their surrounding environment. The mutual signaling controls the secretion and function of many intracellular and extracellular factors like TGF-β (Verrecchia and Rédini, 2018). In 1982, TGF-β was initially described (Anzano, 1982). Now it is established that TGF-βs have a role in the growth arrest of benign neoplasms and in tumor progression and metastasis in the malignant neoplasms (TGF-β paradox) (Zhang, Yu, & Lee, 2014). Our statistical results revealed that the expression of TGF-β1 was detected in both neoplasms. However, TGF-β1 expression was higher in osteosarcoma but without statistically significantly difference.

In classical osteoblastoma, our study revealed that the Tp53 had no expression which is confirmed by the fact that osteoblastoma has very few or no genetic abnormalities (Nord *et al*., 2013). Our results of Tp53 in classical osteoblastoma are in agreement with Oliveira *et al*., 2007 and Chrysomali *et al*., 2011.

Concerning the TGF-β1, its expression was detected in the extracellular stroma. Also, TGF-β1 was detected in osteoblastic rimming layer. To our knowledge, there is no study reporting the expression of TGF-β1 in osteoblastoma. Our observations revealed that there was an immune-negativity of this growth factor in mineralized osteoid tissue. This finding is in agreement with **Kloen *et al*., 1997** who reported that the fixed TGF-β1 in calcified matrix of osteosarcoma becomes unable to bind to the marker protein.

Thus, we can think that the osteoblast - osteoclast interaction in classical osteoblastoma resembles the normal condition. We suggest that the TGF- β may play its normal role as osteoblastic differentiation in

early stage of classical osteoblastoma and as tumor suppressor in the late stage of this tumor. **Zhang et al., 2014** reported that the TGF-β assists cellular homeostasis in benign cells.

Our data revealed that the extracellular matrix showed immunopositivity to TGF-β1, which supports the presence of TGF- β1 in tumor environment of classical osteoblastoma and its role in the migration of mesenchymal stem cells and their differentiation to osteoblasts (Kim *et al*., 2020). **Principe *et al*., 2013** reported that the TGF-β1 can cause growth arrest and apoptosis in benign tumors. Therefore, expression of TGF- β1 in osteoblastic tumor cells means that it may play a role in tumor suppression as there is no role of Tp53.

Therefore, in osteoblastoma there is no disturbed mutual signaling between Tp53 and extracellular molecule TGF-β1. So, we suppose that TGF-β1 acts firstly as osteoblastic differentiating factor at the future site of classical osteoblastoma, then it acts as tumor suppressing factor for this benign bone tumor. Further studies are needed to understood the role of TGF-β1 in classical osteoblastoma.

In the present study, Tp53 was expressed in the nucleus and cytoplasm of osteosarcoma cells, these results are in agreement with **Hu *et al, 2010***. **Chen *et al.,* 2016**, reported that the Tp53 was mutated in osteosarcoma and **Xue *et al.,* 2007** stated that mutated Tp53 may play a role in tumor aggressiveness. Therefore, we can use Tp53 as a diagnostic marker to distinguish between osteoblastoma and osteosarcoma

In the current study, we observed that the TGF -β1 was detected in extracellular stroma of osteosarcoma. Also, there was an immune-positivity of TGF-β1 in osteoclasts, endothelial and perivascular cells of blood vessels, these results are in agreement with **Kloen *et al*., 1997**. Our observations revealed that there was an immune-negativity of this growth factor in mineralized osteoid tissue, however non- mineralized osteoid tissue had an immune-positivity for TGF-β1. Our findings are in agreement with **Kloen *et al*.**, 1997 who reported that the fixed TGF-β1 in calcified matrix becomes unable to bind to the marker protein.

The presence of TGF-β1 in osteosarcoma cells and matrix support the mutual signaling and an autocrine/paracrine cycle (Verrecchia, and Rédini, 2018). The role of TGF-β1 in angiogenesis and metastasis is supported by an immune-positivity in endothelial and perivascular layers of blood vessels (Lamora *et al*., 2016). Therefore, increasing the secretion of TGF-β1 leads to increased proliferation, angiogenesis and metastasis of cancer cells. Thus, we can consider TGF-β1 as a prognostic factor for osteosarcoma.

Interestingly, in our study, there was a moderately negative correlation between expression of Tp53 and TGF-β1 in osteosarcoma. **Hu *et al*., 2010** reported that there was a negative correlation between Tp53 and metastasis of osteosarcoma. High expression of TGF-β1 is associated with poor prognosis (Ma, Zhang, & Li, 2020).

Thus, in osteosarcoma the mutual signaling between osteosarcoma cell and surrounding non-neoplastic cells based on disturbed mutual signaling between Tp53 and TGF-β1. Apoptotic function of TGF-β1 is lost in repression function of Tp53 (Principe et al., 2013). Therefore, loss of tumor suppression effect of both proteins is playing a role in progression of osteosarcoma (Wilkinson, Ogden, & Stratton, 2005). Based on our study, the interaction pathways between Tp53 and extracellular molecule TGF-β1 may play a role in mystery of TGF-b paradox. Further studies are needed to confirm this.

CONCLUSION

The mutual signaling is the main pillar in progression of osteoblastoma and osteosarcoma. The mutual signaling depends mainly on matrix TGF-β1 paradox. In osteoblastoma, there is no role of Tp53, the TGF-β1 may play its normal role as osteoblastic differentiation and then as tumor suppressor. While in osteosarcoma the mutated

Tp53 may lead to aggressiveness in osteosarcoma by changing the paradox of TGF-β1 to the side of protumorigenic activity. Interaction pathways between Tp53 and TGF-β1 may have a role in TGF-β paradox. Understanding this interaction may help in improving of the management and prognosis of osteosarcoma.

REFERENCES

1. Adorno, M., Cordenonsi, M., Montagner, M., Dupont, S., Wong, C., Hann, B., … & Piccolo, S. (2009). A mutantp53/Smad complex opposes p63 to empower TGFβ-induced metastasis. Cell, vol. 137, no. 1, pp. 87–98. https://doi.org/10.1016/j.cell.2009.01.039
2. Anzano, M.A., Roberts, A.B., Meyers, C.A., Komoriya, A., Lamb, L.C., Smith, J.M., & Sporn, M.B. (1982). Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells. Cancer Res, 42:4776–8.
3. Bertin, H., Gomez-Brouchet, A., & R´edini, F. (2020). Osteosarcoma of the jaws: An overview of the pathophysiological mechanisms. Critical Reviews in Oncology / Hematology, 156, 103126. https://doi.org/10.1016/j.critrevonc.2020.103126
4. Chen, Z., Guo, J., Zhang K., & Guo, Y. (2016). TP53 Mutations and Survival in Osteosarcoma Patients: A Meta-Analysis of Published Data. Hindawi Publishing Corporation Disease Markers, vol, Article ID 4639575. http://dx.doi.org/10.1155/2016/4639575
5. Chrysomali, E., Schoinohoriti, O., Theologie-Lygidakis, N., Goutzanis, L., & Iatrou, I. (2011). Osteoblastoma of the mandible: a case report with immunohistochemical evaluation. Open Journal of Stomatology, 1, 207-211. http://dx.doi.org/10.4236/ojst.2011.14032 http://www.SciRP.org/journal/ojst/
6. Elston, R., & Inman, G. J. (2012). Crosstalk between p53 and TGF-β Signalling. Hindawi Publishing Corporation Journal of Signal Transduction, vol, Article ID 294097. https://doi.org/10.1155/2012/294097
7. Goh, A.M., Coffill, C.R., & Lane, D.P. (2011). The role of mutant p53 in human cancer. Journal of Pathology, vol. 223, no. 2, pp. 116–126. https://doi.org/10.1002/path.2784
8. Hu, X., Yu, A.X., Qi1, B.W., Fu, T., Wu, G., Zhou, M., … & Xu, J. (2010). The expression and significance of IDH1 and p53 in osteosarcoma. Journal of Experimental & Clinical Cancer Research, 29:43. https://doi.org/10.1186/1756-9966-29-43
9. Janssens, K., Dijke, P., Janssens, S., Hul, W. (2005). Transforming Growth Factor-B1 to the Bone. Endocrine Reviews 26(6):743–774. https://doi.org/10.1210/er.2004-0001
10. Kabiraj, A., Gupta, J., Khaitan, T., & Bhattacharya, P.T. (2015). Principle and Techniques of Immunohistochemistry – A review. Int J Biol Med Res.;6(3):5204-5210.
11. Kasagi, S., and Chen, W. (2013). TGF-beta1 on osteoimmunology and the bone component cells. Cell & Bioscience, 3:4. http://www.cellandbioscience.com/content/3/1/4
12. Kim, J.M, Lin, C., Stavre, Z., Greenblatt, M.B, & Shim, J.K. (2020). Osteoblast-Osteoclast Communication and Bone Homeostasis. Cells, 9, 2073; https://doi.org/doi:10.3390/cells9092073
13. Kloen, P., Gebhardt, M., Perez-Atayde, A., Rosenberg, A., Springfield, D., Gold, L., & Mankin, H. (1997). Expression of transforming growth factor-beta (TGF-beta) isoforms in osteosarcomas. American Cancer Society. https://doi.org/10.1002/(SICI)1097-0142(19971215)80:12<2230::AID-CNCR3>3.0.CO;2-Y
14. Lamora A., Talbot J., Mullard M., Le Royer, B., Redini, F., & Verrecchia, F. (2016). TGF-β Signaling in Bone Remodeling and Osteosarcoma Progression. Journal of Clinical Medicine. https://doi:10.3390/jcm5110096.
15. Llewellyn, B.D. (2009). Nuclear staining with alum-hematoxylin. Biotech. Histochem. 84: 159-177. https://doi:10.1080/10520290903052899
16. Ma, K., Zhang, C., & Li, W. (2020). TGF-β is associated with poor prognosis and promotes osteosarcoma progression via PI3K/Akt pathway activation. CELL CYCLE. https://doi.org/10.1080/15384101.2020.1805552
17. Mohseny, A., Cai, Y., Kuijjer, M., Xiao, W., Akker, B., Andrea, C., … & Cleton-Jansen, A. (2012). The activities of Smad and Gli mediated signalling pathways in high-grade conventional osteosarcoma. European Journal of Cancer, 48, 3429–3438. http://dx.doi.org/10.1016/j.ejca.2012.06.018
18. Nord, K.H., Nilsson, J., Arbajian, E., Steyern, F., Brosjö, O., Cleton-Jansen, A., … & Hogendoorn, P. (2013). Recurrent Chromosome 22 Deletions in Osteoblastoma

Affect Inhibitors of the Wnt/Beta-Catenin Signaling Pathway. PLOS ONE, vol. 8, Issue 11, e80725. https://doi.org/10.1371/journal.pone.0080725

19. Nakano, K. and Vousden, K. (2001). PUMA, a novel proapoptotic gene, is induced by p53. Molecular Cell, vol. 7, no. 3, pp. 683–694. http://dx.doi.org/10.1016/s1097-2765(01)00214-3

20. Oliveira, C., Mendonça, B., Camargo, O., Pinto, E., Nascimento, S., Latorre, M., Claúdia, M., & Zerbini, N. (2007). Classical osteoblastoma, atypical osteoblastoma, and osteosarcoma. A comparative study based on clinical, histological, and biological parameters. Clinics, 62(2):167-74. http://dx.doi.org/10.1590/s1807-59322007000200012

21. Principe, D.R., Doll, J.A., Bauer, J, Jung, B., Munshi, H.G., Bartholin, L., & Pasche, B. (2013). TGF-β: Duality of Function Between Tumor Prevention and Carcinogenesis. JNCI, vol. 106, Issue 2. https://doi:10.1093/jnci/djt369

22. Sainioa, A., & Järveläinen, H. (2020). Extracellular matrix-cell interactions: Focus on therapeutic applications. Cellular Signaling, 66, 109487. https://doi.org/10.1016/j.cellsig.2019.109487

23. Sanderson, R.D., Elkin, M., Rapraeger, A.C., Ilan, N., & Vlodavsky, I. (2017). Heparanase regulation of cancer, autophagy and inflammation: new mechanisms and targets for therapy. FEBS J. 284;42–55. https://doi.org/10.1111/febs.13932

24. Tang, J., Gifford, C.C., Samarakoon, R., Higgins, P. (2018). Deregulation of negative controls on TGF-beta1 signaling in tumor progression. Cancers;10(6):159. https://doi.org/10.3390/cancers10060159

25. Verrecchia, F., and Rédini, F. (2018). Transforming Growth Factor-β Signaling Plays a Pivotal Role in the interplay Between Osteosarcoma Cells and Their Microenvironment. Frontiers in Oncology. https://doi.org/10.3389/fonc.2018.00133

26. Vousden, K., & Carol, P. (2009). Blinded by the light: the growing complexity of p53. Cell, vol.137. https://doi.org/10.1016/j.cell.2009.04.037

27. Wilkinson, D.S., Ogden, S.K., & Stratton, S.A. (2005). A Direct Intersection between p53 and Transforming Growth Factor β Pathways Targets Chromatin Modification and Transcription Repression of the α-Fetoprotein Gen. MOLECULAR AND CELLULAR BIOLOGY, vol. 25 https://doi.org/10.1128/MCB.25.3.1200–1212.2005

28. Xu, X., Zheng, L., Yuan, Q., Zhen, G., Crane, J., Zhou X., & Cao, X. (2018). Transforming growth factor-β in stem cells and tissue homeostasis. Bone Research. 6, Article number: 2 https://doi.org/10.1038/s41413-017-0005-4

29. Xue, C., Haber M., Flemming, C., Marshall, G., Lock, R., ...& Gudkov A. (2007). P53 determines multidrug sensitivity of childhood neuroblastoma. Cancer Res, 67:10351-60. https://doi.org/10.1158/0008-5472.CAN-06-4345

30. Zhang, Q., Yu, N., & Lee, C. (2014). Mysteries of TGF-β paradox in benign and malignant cells. Frontiers in Oncology, vol.4, Article 94. https://doi.org/10.3389/fonc.2014.00094