Original Article

Evaluation of Cyclin D1 Expression in Aggressive and Nonaggressive Central Giant Cell Granuloma of the Jaws

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KEY WORDS
Giant Cell Granuloma;
Cyclin D1;
Immunohistochemistry;
Aggressive;

Received November 2016;
Received in Revised Form June 2017;
Accepted September 2017;

ABSTRACT

Statement of the Problem: Central giant cell granuloma of the jaws is comprised of two types namely aggressive and nonaggressive. Controversy exists regarding the histogenesis of this lesion. Up to now, there are no reliable histologic or molecular methods to differentiate aggressive from nonaggressive central giant cell granuloma of the jaw. Moreover, because of different treatment of two groups, correct diagnosis is needed.

Purpose: The purpose of this study was to evaluate and compare the expression of cyclin D1 between aggressive and nonaggressive central giant cell granulomas of the jaws.

Materials and Method: This retrospective study was performed on 16 paraffin blocks of aggressive central giant cell granuloma, and 16 nonaggressive central giant cell granulomas from Shahid Beheshti Oral Pathology Department and evaluated the expression of cyclin D1 on giant cells and mononuclear cells of the lesions. T-test was used for quantitative evaluation and comparison of cyclin D1 expression between two groups.

Results: Overexpression of cyclin D1 in giant cells and mononuclear cells of the lesions of both groups was apparent, but no significant statistical difference was seen. Cyclin D1 positivity was seen predominantly in the nuclei of giant cells. When a giant cell was positive, all the nuclei showed immunoreactivity. In each group mean percentage of the positive giant cells were higher than positive mononuclear cells and significant statistical difference (p= 0.000) was seen between them.

Conclusion: Probably overexpression of cyclin D1 implicates in the pathogenesis of the central giant cell granulomas but it seems that this protein could not be used as a marker for identifying the clinical behavior of these lesions.

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Cite this article as: Atarbashi Moghadam S., Ghorbanpour M. Evaluation of Cyclin D1 Expression in Aggressive and Nonaggressive Central Giant Cell Granuloma of the Jaws. *J Dent Shiraz Univ Med Sci.,* 2018 December; 19(4):253-258.

Introduction

The term giant cell lesion (GCL) describes a group of intraosseous nonodontogenic benign lesions containing multinucleated giant cells (GCs). This category of the lesions comprises several entities in the jaws, including brown tumor of hyperparathyroidism, cherubism and the central giant cell granuloma (CGCG). [1]

To date, controversies exist regarding the histogenesis of CGCGs of the jaws. Some researchers believe that this lesion represents a reactive lesion and because of the locally aggressive behavior of some lesions, the others accept it as a benign neoplasm. [2-3]

Most CGCGs of the jaws occur in females before age 30. Approximately 70% of cases arise in the mandi-

ble. [2] Based on clinical and radiographic features, CGCGs can be categorized as aggressive or nonaggressive. [4] The aggressive type has three of five criteria defined as rapid growth, cortical bone thinning, or perforation, root resorption, and tooth displacement. Additionally, a CGCG greater than 5 cm or the lesions with greater recurrence potential after enucleation and curettage is classified as aggressive. The nonaggressive lesion, which comprises most cases of CGCGs, is often asymptomatic, grows slowly, and has a lower rate of recurrence. [4] Thus far, there are no reliable histologic or molecular methods to distinguish the two types from each other. [3-4]

Microscopically, these lesions are characterized by the presence of numerous multinucleated giant cells in a fibrocellular stroma of ovoid to spindle-shaped mononuclear cells (MCs). Foci of hemorrhage with hemosiderin pigment and newly formed osteoid or bone are occasionally observed. [5]

Previous studies have demonstrated the expression of the cell cycle protein Ki-67 in the mononuclear cells of CGCGs, which shows the proliferative activity in these cells. [6-7] This increases the possibility that deregulation of the cell cycle may play a role in pathogenesis of CGCGs.

The cell cycle comprises an ordered series of events that control defined cell cycle stage checkpoints and ultimate cell division. These events are regulated by the expression and degradation, activation and inactivation, and subcellular localization of cyclins and cyclin-dependent kinases (CDKs). [8-10] More than 15 cyclins have been identified, cyclins D, E, A, and B which appear sequentially during the cell cycle and bind to one or more CDK. [11] Genes encoding D-type cyclins (D1, D2, and D3) are induced by mitogenic stimuli. [12] In mammalian cells, CDK4 and CDK6 associate with D-type cyclins and regulate G1 cell cycle phase progression. [8-10, 13-14]

Cyclin D1 is a member of cyclin protein family which is encoded by CCND1 (Cyclin D1 gene) gene on chromosome 11 and regulates the G1/S phase. CDK binds to cyclins and acquires catalytic activity. Activated CDKs in these complexes drive the cell cycle by phosphorylating proteins that are critical for cell cycle transitions. One such protein is the retinoblastoma susceptibility (RB) protein, which normally prevents cells from replicating by forming a tight, inactive complex with the transcription factor E2F. Phosphorylation of RB causes its release, which activates E2F and allows it to stimulate transcription of genes whose products drive cells through the cycle. [11]

Because D-type cyclins provide the link between mitogenic signals and activation of the cell cycle [12, 15] and because of their regulatory function in the G1-to-S transition, [16] constitutive activation of the D-cyclin pathway can overcome or reduce specific mitogen requirements for cell proliferation [17] and thereby contribute to oncogenic transformation. [18-20] Therefore, the purpose of this study was to evaluate and compare the expression of cyclin D1 between aggressive and nonaggressive CGCGs of the jaws to define whether the neoplastic nature of the aggressive lesions.

Materials and Method

Case selection and demographic data of the cases

This retrospective study examined the records and tissue (paraffin blocks) from patients with histopathologic diagnosis of CGCG of the jaws at the Department of Oral and Maxillofacial pathology, Dental School of Shahid Beheshti University of medical sciences, Tehran, from 2000 to 2013. First, according to the clinical, radiographic, and histopathologic records, GCLs of patients with brown tumor of hyperparathyroidism and cherubism were separated from the CGCG. Then, all cases with incomplete or unavailable clinical/ radiographic data and inadequate tissue for sectioning and staining were excluded. All slides were reviewed by an oral and maxillofacial pathologist to confirm the diagnosis.

Clinical data collected included gender and age of the patient, location of the lesion, presence, or absence of pain, cortical expansion, thinning or perforation, root resorption, tooth mobility or displacement, size of the lesion and recurrence. Along with the classification system formalized by Chuong and Kaban, [4] CGCGs were divided into two groups defined as aggressive and nonaggressive. Ultimately, 16 aggressive CGCG and 16 nonaggressive CGCG were studied.

Immunohistochemistry

Sections with 4μm thickness were cut from the paraffin-embedded blocks and mounted on adherent glass slides. Then deparaffinized in xylene and rehydrated in graded

Table 1: Demographic data of the study cases

Lesion	N	Gender		Age				Location		
		Male	Female	Min	Max	Mean age	Std. Deviation	Maxilla	Mandible	Bimax
Aggressive CGCG	16	7(43.8%)	9 (56.3%)	5	35	20.93	8.08	5(31.3%)	10(62.5%)	1(6.3%)
non-aggressive CGCG	16	6(37.5%)	10(62.5%)	5	71	26.18	16.97	8(50%)	8(50%)	0(.0%)
Total	32	13(40.6%)	19(59.4%)					13(40.6%)	18(56.2%)	1(3.1%)

ethanol. Endogenous peroxidase activity was blocked by using 0.5% hydrogen peroxide for 5 minutes, followed by two washes in phosphate-buffered saline solution (PBS) for 5 minutes each. Slides were immersed in deionized water and then rinsed in PBS. Before staining with cyclin D1, antigen retrieval techniques were used. Sections were then incubated with duration and temperature specified for the marker with the primary antibody: cyclin D1 protein-prediluted (Monoclonal mouse Anti-Human anti-cyclin D1, Clone: DCS-6, Code No: M 7155, Denmark, Dako). This was followed by two washes in PBS, incubation with a secondary antibody (Envision Plus; Dako) and 2 PBS rinses. For visualization of immunoreactivity, DAB (3,3'-diaminobenzidine) was used. Then the sections were counterstained with hematoxylin and coverslipped.

Sections of tonsillar tissue served as positive control to verify binding of antibodies to cyclin D1 and as negative control, slides were stained with omission of the primary antibody.

Immunostained sections were assessed and quantified by two investigators blinded to the clinical data for each case. Immunohistochemical reactivity of the multinucleated GCs and stromal mononuclear cells for cyclin D1 were scored on a scale from 1+ to 3+ in ten random selected fields at 400X magnification. Cases with 0-5% positive cells were scored 1+, 6-50% positive cells were scored 2+, and more than 50% were scored 3+. Immunopositivity in more than 5% of cells was considered indicative of overexpression. [21-22]

Statistical analysis

Student's t-test was used to compare the expression of cyclin D1 between two groups. Statistical analyses were performed using SPSS version 20 software. The *p* value of less than 0.05 was considered significant.

Results

Considering the inclusion and exclusion criteria mentioned above, thirty-two cases of central giant cell granuloma were retrieved consisting of 16 aggressive and 16 nonaggressive CGCGs.

Table 1 shows the age and gender of patients and location of the lesions in the two groups. The average age of patients in aggressive and nonaggressive CGCG group were 20.93±8.08 and 26.18±16.97 (mean±SD), respectively. In both groups, female predilection was seen. The mandible was the most common site of involvement in aggressive group. In nonaggressive group, frequency of lesions between two jaws was equal. The percentage and score of GCs and MCs showing immunopositivity for cyclin D1 are summarized in Table 2.

In the present study, overexpression of cyclin D1 was seen in all cases (32/32). Cyclin D1 positivity was seen predominantly in the nuclei of giant cells which had score 3+ in both groups. In aggressive CGCGs positivity of mononuclear cells of 50% of cases were in score 2+ and 50% in score 3+, while in nonaggressive CGCGs 68.8% were in score 2+ and 31.25% in score 3+. When a giant cell was positive, all the nuclei showed immunoreactivity.

Figure 1a and b demonstrates Cyclin D1 protein expression in aggressive and non-aggressive CGCGs, respectively. In both cases, the expression is seen predominantly in the nuclei of giant cells than mononuclear cells.

In both groups mean percentage of the positive GCs were higher than positive MCs (Table 3). Signific-

Table 2: Immunohistochemical Staining Data for Cyclin D1

Lesion	Cell Type	Extent of Immunopositivity for Cyclin D1		
		1+	2+	3+
Aggressive CGCG	GC	0 (0%)	0 (0%)	16 (100%)
	MC	0 (0%)	8 (50%)	8 (50%)
Non-aggressive CGCG	GC	0 (0%)	0 (0%)	16 (100%)
	MC	0 (0%)	11 (68.8%)	5 (31.2%)

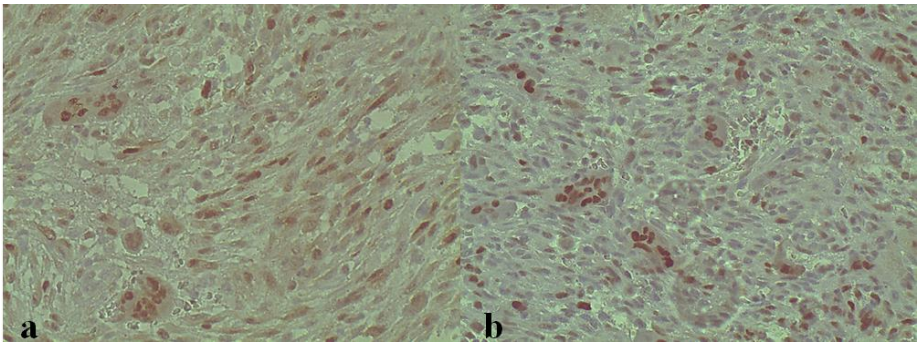

Figure 1a: Cyclin D1 protein expression in aggressive CGCG, (400X), the expression is seen predominantly in the nuclei of giant cells than mononuclear cells **b:** Cyclin D1 expression in non-aggressive CGCG (X400). The expression is seen predominantly in the nuclei of giant cells than mononuclear cells

ant statistical difference (p= 0.000) exists between percentage of GCs and MCs in each group. Comparison of the extent of immunoexpression of cyclin D1 between GCs of two groups (p= 0.656) and MCs of two groups (p= 0.601) was performed using Student's t-test in which no significant statistical difference was seen. Distribution of the giant cells (diffuse or focal) throughout the lesion, as well as size and number of the nuclei of the giant cells had no effect on the pattern of the expression of this protein.

Discussion

In this report, all cases (32/32) demonstrated the overexpression of cyclin D1. The results of this study are in agreement with most of the previous studies. [21-24]

In the recent study, the mean percentage of expression of cyclin D1 in GCs of both groups was greater than MCs. When a giant cell was positive, all the nuclei showed immunoreactivity. This is in line with the results of Matsubayashi *et al.*, Kandel *et al.*, and Kauzmans *et al.* studies. [21-24]

According to the results of different studies, [21-24] it seems that cyclin D1 might play a role in the production of giant cells in these lesions.

In Kauzman *et al.* [22] study, cyclin D1 protein overexpression was present in 28 of 29 cases, and like our study, the GCs had higher expression. They also evaluated this protein on the granulomatous lesions and interestingly found that all the nuclei of GCs were negative for cyclin D1. Therefore, they suggested that the mechanism(s) regulating the formation of GCs in reactive lesions may differ from those in CGCGs and this issue supports the neoplastic theory for CGCGs. In contrast to cyclin D1, evaluation of other cell cycle proteins such as cyclin B1 (showing transition from the G2 to the M phase of the cell cycle) and Ki-67 (which is expressed in all phases of the cell cycle but not in quiescent cells) demonstrated that only mononuclear cells were positive. Moreover, giant cells were negative for these markers indicating that giant cells were not proliferative cells and the expression of cyclin D1 in these cells do not contribute to the proliferative activity .It is involved in the formation and pathogenesis of giant cells. [22] Similar results about the expression of cyclin D1 in giant cell tumors of the bone [21] are also obtained which verifies that CGCG is a tumor.

De Souza *et al.* [25] also investigated the proliferative markers MDM2 and p53 and found MDM2 positive only in mononuclear cells and p53 was negative in both cells (GCs and MCs). As stated by other researchers, [5, 7, 22] the expression of Ki-67 and PCNA in mononuclear cells demonstrates that these cells are proliferative cells in CGCLs. In our study, the expression of cyclin D1 in two groups showed no significant difference. Moreover, O'Malley *et al.* [6] on evaluation of markers of cell cycle such as p53 and Ki-67 (which are involved in proliferation of cells), found no correlation with aggressiveness of the lesions. In their study, p53

Table 3: Mean percentage of Cyclin D1 immunoreactivity

Lesion		N	Minimum	Maximum	Mean	Std. Deviation
Aggressive CGCG	CyclinD1.GC.percent	16	60.80	100	96.61	10.04
	CyclinD1.MC.percent	16	8.30	94.10	51.93	34.03
Non-aggressive CGCG	CyclinD1.GC.percent	16	76.60	100	96.39	7.43
	CyclinD1.MC.percent	16	10	99.10	41.27	32.89

was rarely expressed and giant cells were negative for p53. The extent of Ki-67 immunoexpression was 5.1% and 5.6% in aggressive and nonaggressive lesions respectively. [6]

Kruse-Losler et *al.* [26] found no correlation with aggressiveness and expression of p53 and Ki-67.

In previous studies, [21-22] it has been seen that although overexpression of cyclin D1 occurs in GCs, but they are not proliferating cells. This issue is somewhat unexpected because cyclin D1 is involved in the initiation of cell cycle.

According to Kandel *et al.*'s study, [23] there is accumulating evidence showing that cyclin D1 can have dual functions in cell cycle regulation. [23]It has been shown that elevated levels of cyclin D1 can inhibit proliferation by binding to PCNA. Elevated level of p21 is also contributed to the decrease in proliferation of giant cells. [23]Interestingly, p21 has been implicated in osteoclast differentiation therefore; it is possible that the increased p21 plays a role in regulating differentiation towards multinucleated giant cells in giant cell tumors. [23]

Although Nogueria *et al.* [28] found no statistically significant difference in CCND1 gene amplification between aggressive and nonaggressive CGCGs, amplification of this gene could indicate that CGCL may be true neoplastic in nature. Therefore, these lesions do not support the concept of either reactive or neoplastic process; instead, CGCLs exhibit features of both. [27] It seems that CGCLs may develop in two different ways, one being a reactive process and other appears to be a true benign neoplasm, and that CCND1 amplification may contribute to the tumorigenesis of CGCLs. [28]

In the present study, overexpression of cyclin D1 was seen in all cases in both GCs and MCs; however, the expression was higher in GCs than MCs, which is consistent with the results of other studies. [21-22]

In Kauzman *et al.* study, [21] overexpression of cyclin D1 and cyclin D3 in GCs of giant cell tumor was not accompanied by Ki-67 or cyclin B1 expression. Two possible theories may explain the aberrant expression of cyclins D1 and D3 in the giant cells of giant cell tumor. First, it may show a dysregulation of cell cycle, because of an arrest in G1/S transition, which inhibits the cells from entering the M phase (as evidenced by the absence of cyclin B1 expression). However, if this is the mechanism, it is not evident why there is no Ki-67 expression detected in the giant cells, as this protein is expressed in all cycling cells. Another theory is that cyclin D1 may play a role in giant cell formation. The observation that the percentage of giant cells showing cyclin D1 immunopositivity varies within the tumors does not rule this out because this might be due to differences in the length of formalin fixation of the tissue, in tumor sampling, or in the stage of giant cell maturation. Cyclin D1 protein overexpression has been shown to be associated with giant cell formation, multinucleation, and increased ploidy in different cell models. [21]

Conclusion

Probably overexpression of cyclin D1 implicates in the pathogenesis of the CGCGs but because of no significant difference in expression of cyclin D1 between two groups, it seems that this protein could not be used as a marker for identifying the clinical behavior of these lesions. Therefore, additional studies are required to confirm the role of cyclin D1 and other cell cycle proteins in the pathogenesis of these lesions and to differentiate between aggressive and nonaggressive lesions.

Acknowledgments

The authors would like to thank Oral Pathology Department of Shahid Beheshti University of Medical Sciences and Office of Research of Shahid Beheshti University of Medical Sciences for funding and supporting the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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