Frequent Upregulation of Cyclin D1 and p16^{INK4a} Expression with Low Ki-67 Scores in Multinucleated Giant Cells

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Key Words

Cyclin D1 · p16^{INK4a} · Ki-67 · Multinucleated giant cells

Abstract

Background/Aims: Multinucleated giant cells are formed from the fusion of macrophages and are classified into foreign body-type giant cells (FBGCs), osteoclast-type giant cells (OCGCs) and Langhans-type giant cells (LHGCs). OCGCs display upregulated cyclin D1 expression with low Ki-67 activity. However, little is known about the expression of cell cycle regulators in the other types of multinucleated giant cells. We aimed to investigate the cell cycle status of multinucleated giant cells. **Methods:** The immunohistochemical expressions of cyclin D1, p16^{INK4a} and Ki-67 were analyzed in a total of 127 cases showing multinucleated giant cells. **Results:** Cyclin D1 was overexpressed in 45 (88%) of 51 FBGC cases, 25 (86%) of 29 OCGC cases and 22 (47%) of 47 LHGC cases. p16^{INK4a} showed diffuse nuclear and/or cytoplasmic overexpression in 45 (88%) of 51 FBGC cases, 27 (93%) of 29 OCGC cases and 24 (51%) of 47 LHGC cases. Ki-67 immunostaining was negative in almost all FBGC, OCGC and LHGC cases. **Conclusion:** This study demonstrates that FBGCs and OCGCs frequently show upregulation of cyclin D1 and p16^{INK4a} expression with low Ki-67 scores. This suggests that multinucleated giant cells are arrested in the G1/S cell cycle transition.

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Introduction

Multinucleated giant cells are formed from the fusion of monocyte-derived macrophages at sites of inflammation due to persistent microbial infection, the presence of foreign materials or unknown causes such as sarcoidosis and various tumors [1–4]. Macrophage fusion may lead to foreign body-type giant cells (FBGCs), osteoclast-type giant cells (OCGCs) or mycobacterium-induced Langhans-type giant cells (LHGCs), depending on the known or unknown inciting causes. A variety of molecules including β 1- and β 2-integrin receptor families, interleukin-4, vitronectin, E-cadherin, dendritic cell-specific transmembrane protein (DC-STAMP), P2X7 receptor, CD44 and connexin 43 have been identified as mediators of FBGC formation [5, 6], some of which have also been implicated as common molecules necessary for the multinucleation of both OCGCs and LHGCs [7–9]. However, little is known about the signals that drive multinucleated giant cell formation and maintenance, the molecular mechanism by which macrophage fusion occurs, or their physiological significance at sites of chronic inflammation.

Recently, alterations of cell cycle regulatory proteins, especially those that regulate the G1 to S cell cycle transition, have been suggested in the pathogenesis of bone giant cell tumors [10–12]. The OCGCs of bone giant cell

tumors potentially express cyclin D1 and cyclin D3, but not cyclin B1 and Ki-67 [11]. On the other hand, p21 protein, a cell cycle inhibitor, may assist in cyclin D1 accumulation leading to OCGC formation [10]. Nuclear colocalization of cyclin D1 and β -catenin has been observed in multinucleated giant cells [12]. Since Ki-67 expression is downregulated in multinucleated OCGCs, it has been suggested that cyclin D1 plays an unpredicted role different from the promotion of cell cycle progression. Cell cycle studies on multinucleated giant cells may help understanding giant cell formation and maintenance. However, the expression of cell cycle regulators in FBGCs or LHGCs has not been studied.

In this study, to investigate a relationship between cell cycle regulators and multinucleated giant cells, we analyzed immunohistochemical expressions of cyclin D1, p16^{INK4a} and Ki-67 in different tissue sections with multinucleated giant cells including FBGC, OCGC or LHGC.

Materials and Methods

Tissue Samples

A total of 127 cases showing multinucleated giant cells were selected from the archives of the Department of Pathology, Korea University Ansan Hospital from 2006 to 2010. All tissues were fixed in 10% buffered formalin, embedded in paraffin, and 4 μ m-thick sections were cut. Each section was stained with hematoxylin and eosin. The selected cases (51 FBGC, 29 OCGC and 47 LHGC) were reclassified by consensus of two pathologists (J.-W.C. and Y.-S.K.; table 1). FBGC causative substances were remnant suture material from a previous surgery, implanted medical device, intradermal filler, feces in the anal fistulous tract and endogenous keratinous material ruptured from various kinds of cysts lined by squamous epithelium. OCGC cases consisted of 27 giant cell tumors of tendon sheath and two giant cell tumors of bone. All 47 cases with LHGC were tuberculosis and each section showed chronic caseating granulomatous inflammation. The diagnosis of tuberculosis was confirmed by acid-fast bacilli stain and tuberculosis-polymerase chain reaction.

Immunohistochemistry

Immunohistochemistry was performed using a ChemMate EnVision Kit (Dako, Glostrup, Denmark). Tissue sections were deparaffinized and dehydrated. Sections in 1 mM EDTA buffer (pH 8.0) were heated in a microwave or pressure cooker for 2–10 min. After incubation with 0.3% hydrogen peroxide/methanol for 20 min, slides were stained with anti-cyclin D1 (clone DCS-6; Dako), p16^{INK4a} (clone E6H4, predilution; mtm laboratories AG, Heidelberg, Germany) and Ki-67 (clone MIB-1; Dako) antibodies. After incubation with secondary antibody at room temperature for 30 min, the sections were developed with 3,3'-diaminobenzidine, and counterstained with Harris hematoxylin. Multinucleated giant cells with nuclear and/or cytoplasmic staining were considered positive for p16^{INK4a} and cyclin D1, while multinucleated giant cells with nuclear staining were considered positive for

Table 1. Immunohistochemistry for cyclin D1, p16^{INK4a} and Ki-67 in multinucleated giant cells

	Multinucleated giant cells			p
	FBGCs (n = 51)	OCGCs (n = 29)	LHGCs (n=47)	
Cyclin D1				
0	3 (5.9)	3 (10.3)	15 (31.9)	<0.001
1+	3 (5.9)	1 (3.5)	10 (21.3)	
2+	20 (39.2)	11 (37.9)	15 (31.9)	
3+	25 (49.0)	14 (48.3)	7 (14.9)	
p16 ^{INK4a}				
0	1 (2.0)	0	16 (34.0)	<0.001
1+	5 (9.8)	2 (6.9)	7 (14.9)	
2+	11 (21.6)	7 (24.1)	9 (19.2)	
3+	34 (66.7)	20 (69.0)	15 (31.9)	
Ki-67				
0	36 (70.6)	26 (89.7)	46 (97.9)	0.267
1+	14 (27.5)	3 (10.3)	1 (2.1)	
2+	1 (2.0)	0	0	
3+	0	0	0	

Values are numbers (with percentages in parentheses). Cases with 2+ or more were considered positive; 0 = none; 1+ = less than 5% immunostained cells of total cells; 2+ = 5–50%; 3+ = more than 50%. p values were calculated by the Kruskal-Wallis ANOVA test.

Ki-67. The percentages of immunostained giant cells were semi-quantitatively assessed as: 1+, 0–5% of cells positive; 2+, 6–50% of cells positive; 3+, >50% of cells positive. Cases showing immunopositivity in >5% of cells (2+ or more) were considered positive.

Statistical Analysis

The Pearson χ^2 test and Kruskal-Wallis ANOVA test were used to compare the statistical differences among the three groups including FBGC, OCGC and LHGC. A p value less than 0.05 was considered statistically significant.

Results

Immunohistochemistry results are summarized in table 1. Cyclin D1 overexpression was present in 45 (88%) of 51 FBGC cases (fig. 1a) and 25 (86%) of 29 OCGC cases (fig. 1d), while it was observed in only 22 (47%) of 47 LHGC cases (fig. 1g). Cyclin D1 was evident in the nucleus and/or cytoplasm of the multinucleated giant cells (fig. 1a, d, g). For p16^{INK4a} immunostaining, 45 (88%) of 51 FBGC cases (fig. 1b) and 27 (93%) of 29 OCGC cases (fig. 1e) showed diffuse strong positive staining, whereas only 24 (51%) of 47 LHGC cases (fig. 1h) revealed immunopositivity. The intensity of p16^{INK4a} immunoreactivity

Fig. 1. Immunohistochemistry for cyclin D1, p16^{INK4a}, Ki-67 in multinucleated giant cells. FBGCs expressed strong nuclear or nuclear and cytoplasmic immunoreactivity for cyclin D1 (a) and p16^{INK4a} (b), but not for Ki-67 (c). OCGCs (d–f) and LHGCs (g–i) showed the same immunostaining pattern for cyclin D1, p16^{INK4a} and Ki-67 as FBGCs, although LHGCs infrequently expressed cyclin D1 and p16^{INK4a}. Some mononuclear cells in giant cell tumor of bone (e) were also positive for p16^{INK4a}. ×200.

was stronger in FBGCs and OCGCs than in LHGCs. p16^{INK4a} positivity was found in the cytoplasm or both nucleus and cytoplasm of the multinucleated giant cells, but nuclear staining alone was not observed in any of the cases (fig. 1b, e, h). All FBGC cases except one were nega-

tive for Ki-67 (fig. 1c). All OCGC (fig. 1f) and LHGC (fig. 1i) cases were negative for Ki-67. However, a few giant cells (5% or less) in 14 cases of FBGC were positively stained for Ki-67, but these cases were considered negative according the interpretation criteria of immunohis-

Table 2. Expression patterns of cyclin D1 and p16^{INK4a} in multinucleated giant cells

Cyclin D1/ p16 ^{INK4a}	Multinucleated giant cells			Total (n = 127)
	FBGCs (n = 51)	OCGCs (n = 29)	LHGCs (n = 47)	
+/+	42 (82.4)	23 (79.3)	15 (31.9)	80
+/-	3 (5.9)	2 (6.9)	7 (14.9)	12
-/+	3 (5.9)	4 (13.8)	9 (19.2)	16
-/-	3 (5.9)	0	16 (34.0)	19

Values are numbers (with group percentages in parentheses). Pearson χ^2 test ($p < 0.01$)

tochemistry. Both high expression of cyclin D1 and p16^{INK4a} was found in 42 (82%) of 51 FBGC cases and 23 (79%) of 29 OCGC cases (χ^2 test, $p < 0.01$; table 2). However, this pattern of expression was seen only in 15 (32%) of 47 LHGC cases.

Discussion

In this study, almost all FBGC and OCGC cases displayed upregulated expression of cyclin D1 and p16^{INK4a} with low Ki-67 scores, whereas LHGC often displayed downregulated expression of p16^{INK4a} and cyclin D1. The staining patterns of the cell cycle regulators support the previous observations that FBGC and OCGC share a common mechanism for cell fusion in DC-STAMP^{-/-} mice [8].

The present study revealed that cyclin D1 was overexpressed in FBGC and OCGC. Cytoplasmic staining of cyclin D1 was considered positive because cyclin D1 can accumulate in different subcellular compartments [13, 14] and move across the nuclear pore [15]. Until now, there have been no reports about cyclin D1 expression in FBGC or LHGC. In contrast, the expression of cell cycle regulators has recently been reported in the OCGCs of giant cell tumor of bone [10–12]. Consistent with our results, all OCGCs in giant cell tumor of bone overexpressed cyclin D1 in a manner that was unrelated to its gene amplification [11]. In addition to cyclin D1, it was proposed that p21, a cell cycle inhibitor, binds to cyclin D1 and promotes the formation of multinucleated OCGC [10]. Thus, cyclin D1 might be involved in the formation of multinucleated giant cells, especially OCGCs and FBGCs, although its mechanism remains to be elucidated.

To the best of our knowledge, we first found that p16^{INK4a} expression was upregulated predominantly in FBGC and OCGC. Although p16^{INK4a} inhibits cell cycle progression, the functional significance of p16^{INK4a} in multinucleated giant cells is not clear. p16^{INK4a} binds with cyclin-dependent kinase (CDK) 4/6 to induce an allosteric change that prevents the binding of CDK4/6 to cyclin D1. p16^{INK4a} inhibits CDK4/6-mediated phosphorylation of retinoblastoma family members and promotes retinoblastoma binding with E2F. Thus, p16^{INK4a} blocks the G1/S progression [16]. There has been only one previous report that cellular proliferation suppressors such as p53, p21 and p16^{INK4a} are expressed in both mononuclear cells and multinucleated OCGCs in pigmented villonodular synovitis [17]. In agreement with our study, p16^{INK4a} was expressed in the cytoplasm or both the nucleus and cytoplasm of mononuclear or multinucleated giant cells. Cytoplasmic/perinuclear p16^{INK4a} expression has been demonstrated using immunofluorescence [18]. Since then, the cytoplasmic expression of p16^{INK4a} has been demonstrated in various types of malignancies including non-small cell lung cancer [19], breast cancer [20], high-grade astrocytoma [21] and malignant melanoma [22]. It has been suggested that the cytoplasmic localization of p16^{INK4a} might be a protective response to the overexpression of CDK4.

Consistent with previous reports [11, 12, 23], only a few (<5%) multinucleated giant cells displayed nuclear expression of Ki-67. Given that Ki-67 protein is expressed during all active phases of the cell cycle, this indicates that multinucleated giant cells are arrested at the G1/S transition.

The functional significances for the upregulated expression of cyclin D1 and p16^{INK4a} with low mitotic activity in multinucleated giant cells remain unclear. The present results suggest that alterations of the cell cycle regulator expression may reflect a high metabolic state with limited mitotic divisions in multinucleated giant cells. Contrary to its well-known function of cell cycle progression, cyclin D1 can inhibit cellular proliferation by binding to proliferating cell nuclear antigen, and that forced overexpression of cyclin D1 in normal fibroblasts prevents their entry into S phase [24, 25]. In addition, cyclin D1 decreases cellular adhesion to the extracellular matrix and increases the motility and guided migration of bone marrow macrophages [26].

In summary, the present study demonstrates that cyclin D1 and p16^{INK4a} are overexpressed and Ki-67 is rarely expressed in FBGCs, OCGCs and LHGCs, although LHGCs occasionally expressed cyclin D1 and p16^{INK4a}. This suggests that multinucleated giant cells are arrested

at the G1/S transition. However, the mechanisms by which cyclin D1 or p16^{INK4a} are involved in giant cell formation need to be elucidated. The expression patterns of cell cycle regulators may be useful in detecting multinucleated giant cells, especially FBGCs, OCGCs and LHGCs in histological sections.

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