

Characterization of the common acute lymphoblastic leukaemia antigen (CD10) as an activation molecule on mature human B cells

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SUMMARY

Distinct expression pattern of CD10 molecules during B cell activation was analysed using *in vivo* and *in vitro* systems. By two-colour flowcytometrical analysis, CD10 was found to be expressed at a specific stage of *in vivo* activating B cells. The expression of CD10 during B cell activation appeared to be unique from that of other activation-related B cell antigens including L29, MA6, OKT9 and OKT10. Although the expression of CD10 was associated with that of the activation-related B cell antigens, CD10⁺ B cells could be separated in the distinct fractions to those expressing other activation-related B cell antigens when fractionated by cell gravity. In particular, certain CD10⁺ B cells were detected positive for the resting B cell antigen, L30. *In vitro* studies revealed that CD10⁺ B cells arose from CD10⁻ B cells at an early step of B cell activation, and disappeared lately when activated by *Staphylococcus aureus* Cowan I. Collectively, CD10 was an antigen transiently expressed at an early phase of B cell activation process. Expression of CD10 and other antigens on Burkitt's lymphomas (15 cases) was studied next. All cases were CD10⁺, and 87% (13 cases) were also L30⁺. In addition, six of CD10⁺ L30⁺ cases were L29⁺. This observation suggested that Burkitt's lymphomas were phenotypically similar to the B cells at an early phase of activation, those expressing CD10 and L30, simultaneously. The present study has dissected a precise expression pattern of CD10 on mature B cell activation *in vitro* and *in vivo*, and could be implicated for the histogenesis of one of the poorly characterized B cell lymphoma, namely Burkitt's lymphoma.

Keywords common acute lymphoblastic leukaemia antigen activation antigen Burkitt's lymphoma

INTRODUCTION

Common acute lymphoblastic leukaemia antigen (CALLA) or CD10, originally described as a leukaemia-associated antigen (Greaves & Brown, 1975), is now thought to be the differentiation antigen in a variety of cell lineages. This molecule has especially served to further our understanding of the differentiation pathway of immature B cells (Greaves *et al.*, 1983; Hokland *et al.*, 1984; Ryan *et al.*, 1986; Loken *et al.*, 1987).

Little is known, however, about the role of CD10 as the antigen preferentially expressed on a subset of mature B cells. Recent studies have clearly demonstrated the presence of CD10 on B cells in the germinal center of lymphoid follicles (Gregory *et al.*, 1987; Gadol *et al.*, 1988). The specific distribution of CD10 in peripheral lymphoid tissue may suggest that expression of CD10 is related to cellular activation process because

germinal center is the place for activation and proliferation of B cells (Ishii, Mori & Onoe, 1972).

Recently, various antigens expressed on resting or activated B cells have been reported (Yokochi, Holly & Clark, 1982; Tsudo, Uchiyama & Uchino, 1984; Freedman *et al.*, 1985; Dorken *et al.*, 1986; Kokai, Ishii & Kikuchi, 1986). With the use of such antigens cellular activation pathway has been investigated in detail. Among them, L30 and L29 have been shown to be preferentially expressed on resting and activated B cells, respectively (Kokai *et al.*, 1986). The purpose of this study was to characterize CD10 on mature B cells by comparing with other known B cell antigens.

We describe that CD10 is the activation antigen on mature B cells, inducible on cell surface by *in vitro* stimulation. Furthermore, with the application of these results we describe that Burkitt's lymphomas correspond to the early activated B cells.

MATERIALS AND METHODS

Materials

As a source of normal B lymphocytes, surgically removed

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Table 1. Antibodies used in this study

Antigens	CD	Source/reference
<i>Pan-B cell</i>		
Leu12	CD19	Becton Dickinson, Mountain View, CA
Leu16	CD20	Becton Dickinson
<i>B cell subset (resting B)</i>		
L30		Kokai <i>et al.</i> , 1986
B6, H107	CD23	Nichirei Corp., Tokyo, Japan
2E1	CD32	Cosmo Bio Corp., Tokyo, Japan
B3	CD22	Coulter Immunology, Hialeah, FL
<i>B cell subset (activated B)</i>		
IF6, J5	CD10	Fujimoto <i>et al.</i> , 1988
L29		Kokai <i>et al.</i> , 1986
OKT10	CD38	Ortho Diagnostics, Raritan, NJ
MA6	CD40	Cosmo Bio Corp.
OKT9 (TRFR)		Ortho Diagnostics
Anti-IL-2R	CD25	Becton Dickinson
<i>Others</i>		
Anti-Ig (M, D, K, L)		Becton Dickinson
TdT		Pharmacia Fine Chemicals, Uppsala, Sweden

TRFR, transferrin receptor; IL-2R, interleukin-2 receptor; TdT, terminal deoxynucleotidyl transferase.

hypertrophic tonsils from children were used. Tumour tissues were obtained from children who were diagnosed as having non-Hodgkin's lymphoma (NHL), Burkitt's type (BL) by routine haematoxylin-eosin staining on paraffin sections. Tumour tissues were all collected from the Tokyo area, Japan. Immunohistochemical staining was done on frozen sections according to the method described previously (Ishii *et al.*, 1987).

Monoclonal antibodies (MoAbs)

MoAbs used in this study are listed in Table 1. MoAbs IF6, developed in our laboratory (Fujimoto *et al.*, 1988), and J5 (Ritz *et al.*, 1980) were used to detect CD10 (CALLA). L29 (activated B lymphocytes) and L30 (resting B lymphocytes) were developed in the Department of Pathology, Sapporo Medical College, Japan (Kokai *et al.*, 1986). Biotinylation and FITC conjugation of the purified MoAbs were done according to the methods previously described (Fujimoto *et al.*, 1988). Other commercially available MoAbs and polyclonal antibody listed in Table 1 were also used (Moldenhauer *et al.*, 1987).

Preparation of cell suspensions

Viable mononuclear cells were obtained from minced tissues by centrifugation on a Ficoll-Paque (Pharmacia) gradient ($\rho = 1.017$) (Ishii *et al.*, 1983) and were suspended in RPMI 1640 medium with 10% fetal calf serum (RPMI/FCS). B cell enrichment from tonsil cells was performed by E-rosette depletion as described (Fujimoto *et al.*, 1981). Purity of purified B cell fraction was always more than 93% by immunofluorescence staining with CD19 (Leu12) or CD20 (Leu16). In some experiments, tonsil B cells were further fractionated by Percoll (Pharmacia) density gradient centrifugation into four different fractions (Dagg & Levitt, 1981). Briefly, 90% (v/v) Percoll was made by diluting the Percoll with 1.4 M NaCl, 0.1 M phosphate buffer, pH 7.4. Further dilution was made with 0.14 M NaCl, 0.01 M phosphate buffer, pH 7.4. Discontinuous gradients were

made in a 15-ml centrifuge tube by piling up 2.5 ml of 90%, 70%, 60%, 50% and 40% Percolls sequentially and finally loading $1-5 \times 10^6$ B cells in 1 ml of RPMI/FCS on the top. Tubes were centrifuged at 400 *g* for 40 min and interface fractions were collected (fraction 1, top of 40%; fraction 2, interface at 40% and 50%; fraction 3, interface at 50% and 60%; fraction 4, interface at 60% and 70% of Percoll, respectively).

In vitro activation of tonsil B lymphocytes

One-million B cells per ml were cultured with RPMI/FCS in the presence of 10^{-3} v/v *S. aureus* Cowan I (SACI) (The Enzymatic Center, Malden) and 0.02 ml/ml human interleukin-2 (IL-2) (Japan Antibody Laboratories, Gunma, Japan) at 37°C (Kokai *et al.*, 1986). After cultivation, viable cells separated by Ficoll-Paque gradient were subjected to immunofluorescence staining.

Immunofluorescence (IF) staining

Indirect IF was done according to the method described by Fujimoto *et al.* (1988). As a second reagent, fluorescein isothiocyanate (FITC) conjugated rabbit F(ab')₂ anti-mouse immunoglobulins (Dako, Copenhagen, Denmark) was used at 1:40 dilution. Two-colour IF was performed with the combination of FITC and phycoerythrin (PE). Briefly, after reacting with PE-conjugated and biotinylated antibodies and washing, cells were incubated with FITC-conjugated streptavidin (Becton Dickinson Immunocytometry Systems, Mountain View, CA). As negative controls, class-matched irrelevant MoAbs were used in all experiments. Immunofluorescence was detected by flow cytometer (Epics-Profile, Coulter, Hialeah, FL).

RESULTS

Distribution of CD10 on mature B cells

Distribution of CD10 on purified tonsil B cells compared with other B cell antigens was studied by IF in cell suspension (Table

Table 2. Distribution of various antigens on tonsillar B cells

Antigens	Positivity (%)
CD20	93.3-97.4% (95.8 ± 1.4)
CD10	2.7-43.5% (22.0 ± 11.9)
L30	22.5-88.0% (50.2 ± 16.8)
L29	20.6-64.9% (49.5 ± 12.3)

Mean ± s.d. of 10 samples.

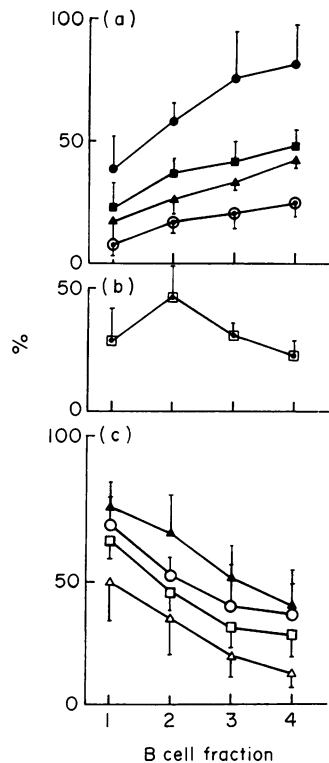


Fig. 1. Phenotype of tonsillar B cells fractionated by cell gravity. For B cell fraction numbers, see Materials and Methods. (a) Resting B cell antigens L30 (●), CD23 (B6, ■), IgD (▲), and CD32 (2E1, ○); (b) CD10 (IF6, □); (c) activation antigens L29 (▲), CD38 (OKT10, ○), transferrin receptor (□), and CD40 (MA6, △). Bar represents mean ± s.d. Note that CD10⁺ B cells were enriched in fraction 2, whereas resting B cell antigens⁺ and activation antigen⁺ cells were enriched in fraction 4 and 1, respectively.

2). On purified B cells, *pan*-B cell antigens such as Leu12 (CD19) and Leu16 (CD20) were always more than 93% positive. CD10 was positive from 2.7 to 43.5% (mean 22.0% of 10 samples) depending on the tissues studied. On the same samples, L30 and L29 were present on 22.5-88.0% (mean 50.2%) and on 20.6-64.9% (mean 49.5%), respectively.

When expression of these antigens was studied on B cells fractionated with cell gravity, interesting results could be obtained. As shown in Fig. 1a, all resting B cell antigens (L30, B6; CD23, IgD and 2E1; CD32) were mostly enriched in B cells

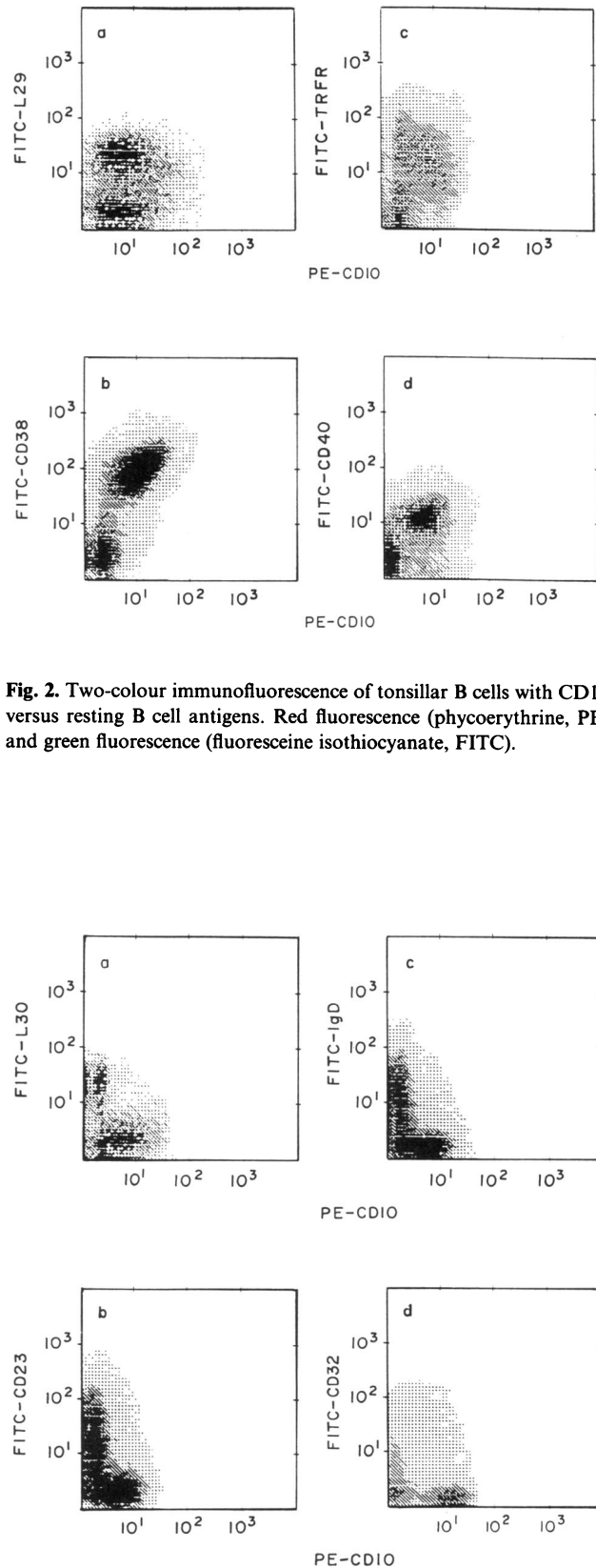


Fig. 2. Two-colour immunofluorescence of tonsillar B cells with CD10 versus resting B cell antigens. Red fluorescence (phycoerythrine, PE) and green fluorescence (fluoresceine isothiocyanate, FITC).

Fig. 3. Two-colour immunofluorescence of tonsillar B cells with CD10 versus activation B cell antigens. Red fluorescence (phycoerythrine, PE) and green fluorescence (fluoresceine isothiocyanate, FITC).

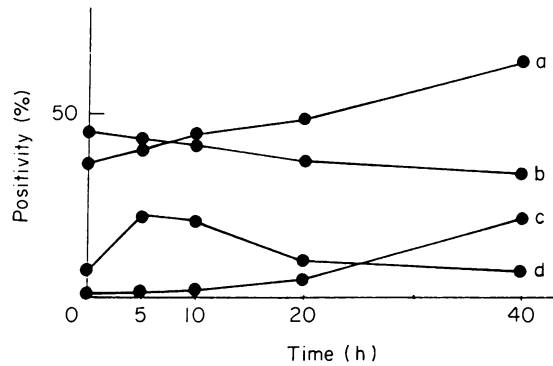


Fig. 4. Appearance of CD10⁺ B cells in *in vitro* culture. Time course of phenotypic change of B cells in *in vitro* activation study is shown. a, L29; b, L30; c, interleukin-2 receptor (CD25); d, CD10.

in fraction 4, the heaviest gravity fraction, and positivities decreased as cell gravity became light. However, all activated B cell antigens (L29, MA6; CD40, OKT9; transferrin receptor, and OKT10; CD38) showed reciprocal distribution to resting B cell antigens. Thus, they were mostly enriched in fraction 1 but positivities decreased as cell gravity increased (Fig. 1c). Interestingly, CD10 showed different distribution from other antigens. As shown in Fig. 1b, CD10⁺ cells increased as cell gravity decreased, the pattern found in activation antigens. However, CD10⁺ cells were mostly enriched in fraction 2 and CD10 positivity decreased in fraction 1. These results clearly indicated that CD10 has a characteristic of activation B cell antigen but that distribution of CD10 is different from other activation B cell antigens.

Two-colour IF analysis of B cells with CD10 and other B cell antigens

When B cells were labelled with CD10 in combination either with L30 or with L29, it was found that CD10⁺ B cells were

mainly L29⁺ (Fig. 2a, upper left) but some CD10⁺ L30⁺ B cells were also identified (Fig. 3a). The relationship between CD10 and other B cell antigens was also analysed. As shown in Figs 2 and 3, most CD10⁺ B cells were found in resting antigens (IgD, CD23, and CD32) negative (Fig. 2), activation antigens (transferrin receptor, CD38, and CD40) positive B cell fraction (Fig. 3).

CD10 expression during *in vitro* activation of B cells

The results described above strongly indicated that CD10 is one of the activation-related molecules on mature B cells. We carried out the *in vitro* activation study in order to find out whether CD10 was a inducible molecule on resting B cells. B cells with scant CD10 positivity were obtained by Percoll gradient centrifugation as described above and were stimulated *in vitro* by SACI. Figure 4 shows the time course of phenotypic change of B cells. B cells were effectively activated since L30⁺ cells decreased and L29⁺ cells increased as stimulation continued. In this system, CD10⁺ cells indeed appeared with quite interesting pattern. Thus, CD10⁺ cells increased from 5 to 25% only 5 h after initiation of culture. However, *in vitro* induced CD10⁺ cells quickly fell down to the initial level after 40 h. In this culture, IL-2 receptor (CD25) appeared in 10 h and gradually increased. Similar results were obtained in repeated experiments (data not shown).

Immunophenotypes of BL

Fresh samples of 15 sporadic BLs were immunophenotyped (Table 3). All cases were positive for CD10 as well as other *pan-B* cell antigens such as CD19, CD20, and immunoglobulins. TdT was negative in all cases. Interestingly, these cases were found to be heterogenous when L30 and L29 were used. Thus, thirteen cases (1–13) were L30⁺ and six cases of them were L29⁺ at the same time. On the other hand, two cases (14 and 15) were L30⁻ but L29⁺. Therefore, with L30 and L29, BLs could be separated into three groups; L30⁺ L29⁻; L30⁺ L29⁺; and L30⁻ L29⁺. No case was positive for CD23 and CD25.

Table 3. Immunophenotypes of fresh Burkitt's lymphoma cases

Case no.	Age (years)	Sex	CD19/20	CD10	sIg	CD38	TRFR	L30	L29	CD23	CD25
1	4	M	+	+	-/κ	+	+	+	-	-	-
2	10	M	+	+	IgM/κ	+	+	+	-	-	-
3	10	M	+	+	IgM/κ	+	+	+	-	-	-
4	10	M	+	+	IgM/κ	NT	NT	+	-	-	-
5	10	M	+	+	IgM/κ	NT	NT	+	-	-	-
6	11	M	+	+	IgM/κ	+	+	+	-	-	-
7	13	M	+	+	IgM/κ	+	+	+	-	-	-
8	4	M	+	+	IgM/λ	-	NT	+	+	-	-
9	6	M	+	+	IgM/-	NT	NT	+	+	-	-
10	8	F	+	+	IgM/κ	+	+	+	+	-	-
11	9	M	+	+	IgM/κ	+	+	+	+	-	-
12	10	F	+	+	IgM/-	+	+	+	+	-	-
13	12	M	+	+	IgM/-	+	+	+	+	-	-
14	10	F	+	+	IgM/κ	-	+	-	+	-	-
15	12	M	+	+	IgM/κ	+	+	-	+	-	-

sIg, surface immunoglobulin; TRFR, transferrin receptor.

DISCUSSION

It has become evident that the distribution of CD10 is not limited to the B cells at early stage of differentiation and their malignant counterparts, common acute lymphoblastic leukemias. CD10 is expressed on uncommitted lymphoid progenitors and its expression decreases as the cells become committed to B or T cells. On mature B cells, CD10 was first reported to be expressed on follicular lymphomas, the tumours derived evidently from the germinal center B cells (Ritz *et al.*, 1981; Greaves *et al.*, 1983). Expression of CD10 on such lymphomas was regarded as an aberrant phenomenon (Ritz *et al.*, 1981; Greaves *et al.*, 1983). Recent studies, however, clearly demonstrated the presence of CD10⁺ mature B cells in germinal centre of lymphoid follicles (Hsu & Jaffe, 1984; Gregory *et al.*, 1987).

The preferential expression of CD10 in germinal centre indicates its potential nature as an activation-related molecule of B cells since germinal centre is the site for activation and proliferation of B cells (Ishii *et al.*, 1972). Close relationship of CD10 to B cell activation was demonstrated in this study by the findings that (i) most CD10⁺ cells are included in *in vivo* activated B cells positive for a variety of activation antigens including L29, transferrin receptor, and CD40; and (ii) CD10⁺ cells emerged from CD10⁻ cells as revealed by *in vitro* activation culture with SACI. However, CD10 has a different characteristic from a variety of activation-related molecules on B cells because CD10⁺ B cells were enriched in the fraction with intermediate cell gravity, whereas other activation antigen positive B cells were enriched in the fraction with the lowest cell gravity. Since cell gravity of lymphocytes are considered to decrease when they are activated, it is likely that CD10⁺ tonsillar B cells corresponded to the B cells at intermediate activated phase. Consistent with this result in *in vivo* activated tonsillar B cells, similar results were obtained in *in vitro* activation experiments. CD10⁺ B cells appeared quickly in culture with SACI but such CD10⁺ cells disappeared in 48 h. In contrast, other activation antigen, such as L29⁺ and CD25⁺ cells continued to increase. Particularly, expression patterns of CD10 and CD25 were quite different, suggesting that these two molecules are expressed on distinct phase of B cell activation process in *in vitro* systems. From these results, we concluded that CD10 is an activation antigen on mature B cells transiently expressed at very early activation phase.

These basic observations, especially on the characterization of CD10, were of particular importance to study the histogenesis of BLs. From a pure histological point of view, BLs have been postulated to originate from germinal centre B cells (Mann *et al.*, 1976). Our study confirmed this by analysing their antigenic phenotypes. Immunophenotypically, they were evidently derived from mature B cells since none of them expressed TdT but all had cell surface immunoglobulins as well as other *pan-B* antigens. By utilizing CD10 and other B cell antigens, BLs were found to have phenotypes of activated B cells. Consistent with our results, Gregory *et al.* (1987) recently reported the identification of normal B cells in the germinal center having identical phenotypes to BLs, i.e. CD10⁺, BLA (Burkitt's lymphoma associated antigen)⁺, Epstein-Barr virus receptor⁺, sIgM⁺ B cells. As described in this study, CD10 is evidently one of the activation-related molecules of B cell differentiation. Therefore, positivity for CD10 in all BLs clearly indicate the activated B cell nature of BLs. Of note was the finding that

CD10⁺ BLs could be separated into three subtypes by utilizing L30 and L29, i.e. L30⁺ L29⁻, L30⁺ L29⁺, and L30⁻ L29⁺. Particularly, the finding that CD10⁺ L30⁺ BLs accounted for the majority of cases was very interesting. During B cell activation, L30⁺ L29⁻ resting B cells were reported to become L30⁻ L29⁺ cells (Kokai *et al.*, 1986). Consistently, NHLs, small cell type, were L30⁺ L29⁻ which correspond to the resting B cells, whereas NHLs, large cell type, were L30⁻ L29⁺ which represent the activated B cells (Ishii *et al.*, 1986). These observations as well as the results in this study strongly suggest that CD10 appears in the course of B cell activation with phenotypic change from L30⁺ L29⁻ to L30⁺ L29⁻. Therefore it is very likely that BLs represent CD10⁺ B cells which appear at a very early stage of activation.

Considering the previous observations and the results described here, CD10 shows a quite unique distribution on B cell differentiation pathway, i.e. progenitor B cells and early activated B cells. Recent study with cDNA coding for CD10 has revealed a striking feature that CD10 cDNA encodes a protein almost identical to rat and rabbit neutral endopeptidase 24.11, enkephalinase, which inactivates a variety of peptide hormones (Shipp *et al.*, 1989). Enzymatic activity of CD10 has also been shown by the CD10⁺ transfectants although it is yet unknown why this enzymatically active molecule is expressed at very specific stages of B cell differentiation. However, the stages at which CD10 is expressed are of particular importance for B cell maturation in bone marrow and clonal proliferation in peripheral organs. These observations underscore the importance of CD10 molecule as a member of functional molecules on lymphocytes and enable us to explore the presence of the unknown mechanism of cell proliferation and differentiation.

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