Cutting Edge: Polycomb Gene Expression Patterns Reflect Distinct B Cell Differentiation Stages in Human Germinal Centers

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Polycomb group (Pc-G) proteins regulate homeotic gene expression in Drosophila, mouse, and humans. Mouse Pc-G proteins are also essential for adult hematopoietic development and contribute to cell cycle regulation. We show that human Pc-G expression patterns correlate with different B cell differentiation stages and that they reflect germineral center (GC) architecture. The transition of resting mantle B cells to rapidly dividing Mib-1(Ki-67)+ follicular centroblasts coincides with loss of BMI-1 and RING1 Pc-G protein detection and appearance of ENX and EED Pc-G protein expression. By contrast, differentiation of centroblasts into centrocytes correlates with reappearance of BMI-1/RING1 and loss of ENX/EED and Mib-1 expression. The mutually exclusive expression of ENX/EED and BMI-1/RING1 reflects the differential composition of two distinct Pc-G complexes. The Pc-G expression profiles in various GC B cell differentiation stages suggest a role for Pc-G proteins in GC development. The Journal of Immunology, 2000, 164: 1–4.

Recent studies identified polycomb group (Pc-G) genes as essential regulators of mouse lymphoid development (1–3). Pc-G proteins form an evolutionary conserved cellular memory system that ensures stable transmission of gene activity (4, 5). These gene-regulatory proteins were originally discovered in Drosophila as repressors of homeobox genes. Pc-G proteins and their trithorax group counterparts, which have an opposing, activating role, ensure correct embryonal development. Mutations in Pc-G genes can result in homeotic transformations, the change of one body part into another (4). Pc-G proteins form heterogeneous multimeric protein complexes (6–11). In humans, two distinct Pc-G complexes were identified by yeast two-hybrid analyses. The BMI-1 Pc-G protein is part of the first complex and coimmunoprecipitates, cofractionates, and colocalizes with RING1, HPH1, HPH2, and HPC2 Pc-G proteins (7, 8, 12, 13). These Pc-G proteins have extensive interactions with each other. A second Pc-G complex contains the ENX and EED Pc-G proteins. ENX and EED interact with each other and coimmunoprecipitate. However, neither ENX nor EED interacts or coimmunoprecipitates with Pc-G proteins of the other complex, such as BMI-1 or HPC2 (9). The composition of these distinct Pc-G complexes probably contributes to target gene specificity and tissue-specific gene expression.

The role of Pc-G genes in developmental processes continues in adult life, and studies of Pc-G mutant mice demonstrated that Pc-G genes are essential for lymphoid development. Lymphocyte precursors in mice with a targeted deletion of MEL-18 (1) or BMI-1 (2) insufficiently respond to IL-7, which ultimately leads to severe lymphoid hypoplasia. Upon activation through their Ag receptor, B cells of MEL-18 transgenic mice enter cell cycle arrest through a c-myc/cdc25 cascade (14). By contrast, BMI-1 transgenic mice exhibit enhanced lymphoproliferation and induction of lymphomas, possibly by suppressing expression of p16/INKA4 and p19arf (15), and in collaboration with c-myc (3, 16). The latter observations demonstrate that Pc-G proteins also have an important role in regulation of the cell cycle.

The contribution of Pc-G genes to regulation of human hematopoiesis is relatively unexplored. PCR analysis of bone marrow subpopulations showed marked changes of human Pc-G transcription levels in developing lymphoid precursors (17). Pc-G transcription is detectable in tissues with high cell division rates, such as embryonic tissue or bone marrow, and appears to change at different stages of differentiation. Since germineral centers (GCs) are characterized by high cell proliferation rates, with maintenance of spatial maturity patterns of lymphoid cells, we questioned whether differentiation of B cells in GCs is reflected in altered expression of human Pc-G genes. We demonstrate that the GC mantle zone, dark zone (DZ), and light zone (LZ) are associated with distinct expression profiles of the BMI-1/RING1 and ENX/EED Pc-G proteins. These patterns reflect the differential composition of the two previously identified distinct Pc-G complexes and suggest that human Pc-G proteins play a role in GC development and Ag-specific B cell differentiation.
Materials and Methods

Tonsils and lymph nodes were obtained freshly from the surgery room, fixed in 4% buffered Formalin, and embedded in paraffin. Sections (3 μm) were cut and deparaffinized, and endogenous peroxidase was inhibited with 0.3% H₂O₂ in methanol. For immunohistochemistry, primary Abs were applied following microwave Ag retrieval and preincubation with swine or goat serum. BMI-1, RING-1, ENX, and EED Pc-G proteins were detected with the 6C9 mouse monoclonal and K320, K358 and K365 rabbit polyclonal antisera, respectively (9). Secondary antisera were biotinylated goat anti-mouse or biotinylated swine anti-rabbit. Immunostaining was performed with 3-amino-9-ethylcarbazole using the streptavidin-biotin-avidin complex/HRP method and tyramine intensification. Sections were counterstained with hematoxylin and photographed with a Zeiss Axiophoto microscope (Zeiss, Oberkochen, Germany).

For double immunofluorescence, tissue sections were fixed in 2% formaldehyde and endogenous peroxidase was inhibited as above. Following preincubation with 5% BSA, primary Abs against BMI-1 (6C9) or Mib-1/Ki-67 were applied in combination with K358 (anti-ENX) or A452 (anti-CD3). BMI-1 and Mib-1 were detected by biotinylated goat anti-mouse antiserum followed by streptavidin-CY3, ENX and CD3 were detected by swine anti-rabbit Ig-peroxidase-antiperoxidase, followed by FITC. Cross-reactivity of the antisera was excluded by appropriate controls, and sections were analyzed and photographed with a Leica DMRB immunofluorescence microscope (Leica, Deerfield, IL).

Results and Discussion

B cell subpopulations in the GC have distinct BMI-1 and ENX expression patterns

GCs are sites of multiplication and selection of Ag-specific B cells and consist of a follicle surrounded by a mantle zone (18, 19). The follicle contains a DZ and a LZ. During the GC reaction, rapidly dividing centroblasts give rise to centrocytes that are subject to antigenic selection. The DZ is primarily composed of centroblasts and centrocytes, whereas the LZ mainly contains centrocytes, a follicular network of Ag-presenting dendritic cells and helper T cells. B cells in the mantle zone are regarded as the precursors of centroblasts (the primary follicle).

We performed an immunohistochemical analysis of Pc-G expression in GCs of the human tonsil. BMI-1 and RING1, and ENX and EED, were investigated as representative of two different human Pc-G complexes. Expression of these proteins was detected in nuclear staining patterns, as shown previously (7–9), and depended on the stage of lymphocyte development in the GC reaction (Fig. 1). Similar results were obtained with GCs in lymph nodes (data not shown). Resting B cells in the mantle zone stained for BMI-1/RING1 with varying intensity, but did not express ENX or EED (Fig. 1, B and C), whereas the vast majority of such cells expressed ENX and EED (Fig. 1, D and E). A large portion of LZ centrocytes expressed BMI-1 and RING1 (Fig. 1, F and G), whereas ENX and EED expression in the LZ was notably reduced (Fig. 1, H and I). Most ENX⁺ or EED⁺ cells in the LZ had a centroblast appearance. Note that occasional BMI-1⁺ or RING1⁺ cells in the DZ were ENX⁻ and EED⁻-tingible body macrophages (Fig. 1, J–L). In addition, a minority of BMI-1⁺ follicular lymphocytes were T cells (Fig. 2A).

The BMI-1/RING1 and ENX/EED staining patterns suggested that expression of these proteins is mutually exclusive during the GC reaction. We therefore detected BMI-1 and ENX by double immunofluorescence to further explore follicular Pc-G expression patterns. As predicted by immunohistochemistry, BMI-1 and ENX expression did not overlap. Mantle zone B cells were single positive for BMI-1 and did not stain for ENX, whereas follicular cells expressed either BMI-1 or ENX (Fig. 2B). Since transition from

FIGURE 1. Immunohistochemical detection of human Pc-G BMI-1, RING-1, ENX, and EED proteins in secondary GCs of the tonsil. A–D, GC overview. E–H, Detail of the DZ and mantle zone (M). I–L, Detail of the LZ and M. A, E, and I, Detection of BMI-1; B, F, and J, detection of RING-1; C, G, and K, detection of ENX; D, H, and L, detection of EED. Centroblasts (cb) contain large, plump nuclei and primarily reside in the DZ. Centrocytes (cc) contain angular or cleaved nuclei and primarily reside in the LZ. Mφ, tingible body macrophage.
resting mantle B cell to follicular centroblasts is associated with a high rate of cell division, we questioned whether ENX high rate of cell division. We therefore likely contributing factors to the switch in Pc-G expression profiles.

Concluding remarks

Pc-G genes are important regulators of cellular differentiation dur-
ing embryonic development and also appear to contribute to reg-
ulation of hematopoiesis. Our analysis of human Pc-G expression dem-
strated that BMI-1/RING1 and ENX/EED expression patterns were mutually exclusive in the GC and dependent on the developmental stage of the B cell. Follicular precursor B cells in the mantle, and follicular centroblasts and centrocytes, exhibited characteristic Pc-G staining patterns that can be interpreted as generation of different Pc-G complexes and consecutive on/off switching of Pc-G gene expression during GC formation. We propose that these gene-regulatory proteins play a role in GC development and Ag-specific differentiation of B cells.

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