Ultrastructural Pathology

Ribosome-Lamella Complex Precursors in Acute Monocytic Leukemia: A Study of 6 Cases

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Ribosome–Lamella Complex Precursors in Acute Monocytic Leukemia: A Study of 6 Cases

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ABSTRACT The ribosome–lamella complex (RLC) is a cylindrical structure composed of annular lamella associated particles, regarded as ribosomes, around a central core, which is best known in hairy cell leukemia. RLC has been presumed to originate from aggregating rER and ribosomes. Incomplete and maturing RLC structures have been called RLC precursors (pre-RLC). The present paper investigates the various architectural aspects of pre-RLC and the ultrastructural characteristics of the blasts in 6 cases of acute monocytic leukemia (M5) in which these structures occur. Blasts bearing pre-RLC contained irregular nuclei with less heterochromatin and a prominent nucleolus, and many cytoplasmic organelles in an abundant cytoplasm. The findings indicate that pre-RLC might result from an asymmetrical differentiation of organelles in blasts associated with expression of CD117 and CD56 but default of CD14 in M5.

KEYWORDS acute monocytic leukemia, precursor, ribosome–lamella complex, ultrastructure

Ribosome–lamella complexes (RLC) have been found in a variety of hematologic malignancies and occasionally in solid tumors [1, 2]. They occur most frequently in hairy cell leukemia (HCL) and have served as a diagnostic marker in the past [1]. Previous observations revealed that RLC was constructed of rER and ribosomes [3]. When RLCs show a variable and incomplete architecture they are referred to as precursors of ribosome–lamella complexes or immature ribosome–lamella complexes [4]. Although the process of development of RLC has been investigated systematically, the characteristics of leukemia cell-bearing pre-RLC have seldom been described in detail. Here, we illustrate the features of developing pre-RLC in 6 cases of M5, whose clinical and laboratory data and ultrastructural features are also reported.

MATERIALS AND METHODS
Clinical Data and Laboratory Examination

Six cases of de novo M5-bearing pre-RLC were identified from 92 patients with M5 occurring in the whole leukemia file in Electron Microscopy
Department of our hospital from 2004 and 2006 (451 cases). The group included 5 males and 1 female; the median age was 22 years, and the range was 15–31 years. Three were diagnosed as M5a and 3 as M5b. All patients received standard chemotherapy. Four patients achieved complete remission (CR), 3 of them relapsed within one year, 1 patient achieved partial remission (PR), and 1 other experienced no remission (NR) (Table 1).

Cytochemistry and immunophenotyping were performed before chemotherapy treatment (Tables 2, 3). The cytochemistry examination was evaluated according to routine procedures [6]. Heparinized samples of bone marrow aspirate (BMA) were analyzed by flow cytometry [7]. Positive markers were defined as the expression of over 20% of blasts for surface antigens, and 10% for cytoplasmic and nuclear antigens. All cases were diagnosed by combining results from morphology, cytochemistry, and immunophenotyping [8, 9].

**Transmission Electron Microscopic Examination**

The electron microscopy was carried out as previously described [10]. Briefly, nucleated cells (10^6 cells) were isolated from 3–5 mL of anticoagulated BMA, fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, then embedded in Epon 812. The ultrathin sections were stained in uranyl acetate

**TABLE 1** Clinical Data in the 6 Cases with M5 Bearing RLC

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Symptoms</th>
<th>Objective sign</th>
<th>Subtype</th>
<th>Result</th>
<th>DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>31</td>
<td>Debility, anemia</td>
<td>Lymphadenopathy</td>
<td>M5a</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>21</td>
<td>Debility, dizziness</td>
<td></td>
<td>M5a</td>
<td>CR</td>
<td>6 months</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>22</td>
<td>Bone pain</td>
<td>Lymphadenopathy skeletal tenderness</td>
<td>M5b</td>
<td>CR</td>
<td>?</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>20</td>
<td>Fever, debility</td>
<td>Lymphadenopathy</td>
<td>M5a</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>15</td>
<td>Fever, debility</td>
<td>Lymphadenopathy splenohepatomegaly</td>
<td>M5b</td>
<td>CR</td>
<td>12 months</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>25</td>
<td>Fever, debility</td>
<td>Lymphadenopathy</td>
<td>M5b</td>
<td>CR</td>
<td>11 months</td>
</tr>
</tbody>
</table>

Note: CD, clinical diagnosis; DFS, disease-free survival; CR, complete remission, PR, partial remission; NR, no remission; ?, unknown.

**TABLE 2** Cytochemistry of 6 Cases with M5 Bearing RLC (positive percentage, index)

<table>
<thead>
<tr>
<th>No.</th>
<th>MPO</th>
<th>SBB</th>
<th>CE</th>
<th>NAE</th>
<th>NAE + NaF</th>
<th>PAS</th>
<th>NBE</th>
<th>ACP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0, 0</td>
<td>80, 82</td>
<td>0, 0</td>
<td>64, 64</td>
<td>0, 0</td>
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<td>0, 0</td>
<td>12, 12</td>
<td>95, 211</td>
<td>24, 24</td>
<td>100, 123</td>
<td>98, 319</td>
<td>54, 55</td>
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<tr>
<td>3</td>
<td>98, 152</td>
<td>100, 170</td>
<td>48, 76</td>
<td>36, 36</td>
<td>16, 16</td>
<td>94, 94</td>
<td>3, 3</td>
<td>32, 32</td>
</tr>
<tr>
<td>4</td>
<td>36, 42</td>
<td>66, 86</td>
<td>0, 0</td>
<td>12, 12</td>
<td>0, 0</td>
<td>100, 114</td>
<td>4, 4</td>
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<tr>
<td>5</td>
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<td>2, 2</td>
<td>18, 18</td>
<td>100, 210</td>
<td>22, 22</td>
<td>100, 142</td>
<td>100, 284</td>
<td>100, 230</td>
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<tr>
<td>6</td>
<td>2, 2</td>
<td>30, 40</td>
<td>77, 82</td>
<td>100, 356</td>
<td>14, 14</td>
<td>100, 163</td>
<td>100, 393</td>
<td>92, 124</td>
</tr>
</tbody>
</table>

Note: SBB, Sudan black B; CE, naphthol AS-D chloroacetate esterase; NAE, α-naphthyl acetate esterase; PAS, periodic acid-Schiff-hematoxylin; NBE, α-naphthyl butyrate esterase.

**TABLE 3** Immunophenotype of 6 Cases with M5 Bearing Pre-RLC

<table>
<thead>
<tr>
<th>No.</th>
<th>HLA-DR</th>
<th>CD34</th>
<th>CD33</th>
<th>CD13</th>
<th>CD117</th>
<th>CD14</th>
<th>CD15</th>
<th>CD64</th>
<th>CD56</th>
<th>CD7</th>
<th>CD19</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>–</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: NT, not tested.
and lead citrate and were examined in a Hitachi H-600 electron microscope. Myeloperoxidase (MPO) activity was measured by Roel's method [11].

RESULTS

Most leukemia cells in the 5 cases demonstrated monoblastic features, with a diameter ranging from 18 to 25 μm, much larger than monoblasts in normal bone marrow. The blasts had a round or irregular shape and a few projections scattered over the surface. The nuclear volumes were large and their outlines often regular; the heterochromatin was less than in a typical promonocyte and the nucleoli were more distinctive (Figure 1). Over half of the leukemia cells showed abundant cytoplasm including plentiful rER, many ribosomes, a moderately developed Golgi apparatus, and a few granules of high electron density.

The pre-RLCs were generally similar in the 6 cases. All pre-RLCs were associated with rER, although the number and location of associated ribosomes as well as the numbers of layers of pre-RLC were different in individual blasts. The associated and unassociated rER cisternae in blasts were uniformly narrow and unexpanded. Some pre-RLC formed complete cylindrical structures consisting of rolled up rER cisternae, which were disconnected from each other (Figure 2). In others, some rolled up rER cisternae were not completely closed into a circle but connected to

FIGURE 1  (A) The features of blasts bearing pre-RLC (arrow), case 1, ×4000. (B–D) pre-RLC in different planes of section, case 1, ×8000.
rER lying outside the cylindrical structures, and therefore showed incomplete concentric cylinders (Figure 3). All incomplete concentric cylinders and a proportion of complete concentric cylinders possessed just layers of rER without free ribosome between them. Alternatively, a small proportion of pre-RLC had diffuse free ribosomes between the rER cisternae, and these pre-RLC were considered to be more mature structures. The concentric cylinders contained cytoplasmic matrix and occasional small mitochondria. The number of pre-RLC correlated with the amount of rER in the blasts and the percentages of blasts bearing pre-RLC were different in the 6 cases: over 50% in 2 out of 6 cases; less than 20% in 2 cases; and an intermediate value for the last 2 cases.

**DISCUSSION**

RLC has been described as a cylinder-like structure comprising lamellae and ribosomes around a central core of cytoplasmic matrix. RLCs are usually 2.2–4.1 μm in length, with a diameter depending on the number of lamellae as well as on the contents of the inter- and intralamellar space, ranging from 0.52 to 1.27 μm [12]. It has been suggested that RLC originates from the aggregation of rER into a concentric configuration, followed by consecutive maturation steps. All of the structures resembling RLC but lacking the typical mature appearance of RLC have been called precursors of RLC or RLC precursors [5].

According to our observations, 6 cases of M5 contained pre-RLC with varying structure, but with a
complete concentric cylinder and 2 or more layers of rER. Some of them connected with flat rER outside the cylinder, confirming the pre-RLC derivation from rER in the cytoplasm. The ribosomes associated with pre-RLC in varying ways. In some pre-RLC, ribosomes attached to rER and no free ribosomes were present between the rER layers, while some pre-RLC had free ribosomes aligned in a circle between and parallel with the rER. Some pre-RLC had fewer ribosomes attached to rER. All of these structures were grouped into the pre-RLC category. The spectrum of architecture constituted a successive developmental process of pre-RLC formation in m5 from rER in a step by step fashion (Figure 4) [13].

Interestingly, monoblasts bearing pre-RLC in 6 cases exhibited some peculiar ultrastructural features. On the one hand, the blasts illustrated differentiated features, such as abundant cytoplasm, overlapping rER, secretory vacuoles and granules, structures usually thought of as promonocyte characteristics. On the other hand, they had large and regular nuclei containing less heterochromatin and a prominent nucleolus, which are features usually found in monoblasts other than promonocytes. Meanwhile, the rER in blasts bearing pre-RLC was uniformly narrow. The characteristics constituted a unique asymmetric differentiation—a more mature cytoplasm but immature nucleus. These features hinted that the pre-RLC

**FIGURE 3** (A) Inside layer of pre-RLC extending out and connecting with “external” rER (arrow), case 5, ×8000. (B) Second layer formation (arrow), case 5, ×8000. (C) Third layer formation (arrowhead), case 3, ×15,000. (D) Associated rER and more mature pre-RLC, case 3, ×20,000.
in blasts of M5 might result from aberrant metabolism of plasma membrane system.

The cytochemical characteristics of the 6 cases were consistent with monocytic features and provided the main basis for diagnosis. Immunophenotypically, all 6 cases were positive for CD33, CD13, and HLA-DR; none were positive for CD14, which is often restricted to M5b [14]. Four cases tested were CD117 positive, which was usually expressed in early myeloid leukemic cells [15]. Four cases were positive for CD56, more frequently than the average level for M5 [16]. The expression of CD56 was thought to be associated with precursors of acute leukemia cells in hematologic malignancies [17]. These characteristics suggest that pre-RLC may be related with CD117- and CD56-positive and CD14-negative expression in M5.

CONCLUSION

The present observations support previous reports in which pre-RLC was constructed of overlapping rER, forming various architectural patterns representing a successive development of RLC. The occurrence of pre-RLC may result from asymmetric differentiation of organelles in blast, and may positively associate with the expression of CD117 and CD56, and negatively associate with the expression of CD14 in M5.

REFERENCES


FIGURE 4 Formation of concentric cylinders from associated rER.