Expression Pattern of the CCAAT/Enhancer-Binding Protein C/EBP-β in Gestational Trophoblastic Disease

Jessica Radde, Thomas Löning, and Ana-Maria Bamberger

Summary: The CCAAT/enhancer-binding protein (C/EBP) family consists of several factors that are important regulators of intracellular processes and hormone action. C/EBP-β, the most important member of the C/EBP family, was shown recently to be expressed in the normal human placenta where it is localized in villous syncytiotrophoblast and in the extravillous (intermediate) trophoblast but not the villous cytotrophoblast. The purpose of this study was to investigate the expression pattern of C/EBP-β in gestational trophoblastic disease (GTD) which has not been studied so far. We used immunohistochemistry on a total of 15 cases of GTD including nine complete hydatidiform moles, one placental site nodule (PSN), one placental site trophoblastic tumor (PSTT), and four choriocarcinomas. All our tested specimens showed positivity for C/EBP-β. The strongest C/EBP-β expression could be observed in villous syncytiotrophoblast and in the trophoblast proliferations on the villous surface of hydatidiform moles; villous cytotrophoblast was negative. The PSN also showed positive nuclear staining but the expression was not as strong as it was in the hydatidiform moles and the total amount of stained cells was the lowest of all GTD. The PSTT also showed immunoreactivity but with a weaker and more heterogeneous staining than in the choriocarcinomas. The specific expression pattern of C/EBP-β in GTD indicate that C/EBP-β could potentially be an additional marker of such lesions. Key Words: CCAAT/enhancer-binding protein—C/EBP-β—trophoblast—hydatidiform mole—trophoblastic tumor—choriocarcinoma.

Implantation and placentation in women involve a series of processes that require tight regulation. The placenta is an autonomous organ responsible for fetal-maternal nutritional and gas exchanges and whose endocrine and immunological functions are essential for fetal growth and for maintaining pregnancy (1, 2). The trophoblastic tissue of the human placenta is unique in its ability to proliferate and invade another tissue in a controlled fashion. Thus it can be regarded as an important model for the study of molecular mechanisms involved in these processes and for differentiating them from those implicated in tumor progression (3).

During development of the human placenta, the stem cell-like cytotrophoblast (CT) proliferates and gives rise to the differentiated syncytiotrophoblast (ST) on the villous surface and to the extravillous (intermediate) trophoblast (IT) that provides the anchoring of the conceptus and the placenta at the maternal-fetal interface (3, 4, 5). The normal development of the trophoblast is crucial for implantation and further survival of the embryo.

The family of the CCAAT/enhancer-binding proteins (C/EBP) are transcription factors acting as basic region/leucine zipper DNA-binding proteins of which six members have been described: C/EBP-α, -β, -δ, -ε, -γ, and -ζ (6–8). The expression pattern of C/EBP-β in the normal human placenta has been investigated and has shown to have the highest expression level of all members of the C/EBP family. A strong positive nuclear staining in villous ST and in the extravillous IT was found while villous CT was negative (9). The specific expression pattern of the most important factor of the C/EBP family, C/EBP-β, in GTD has not been studied so far. Therefore, this study was performed to investigate the expression pattern of C/EBP-β in GTD using immunohistochemistry.
MATERIALS AND METHODS

Tissue Collection

The tissue material was selected following histological review from the files of the Department of Gynecologic Pathology, University Hospital Eppendorf, Hamburg. For immunohistochemistry, specimens that had been routinely fixed in 4% buffered formalin and embedded in paraffin were used. Specimens (n = 15) included 9 cases of complete hydatidiform mole (one with invasion present in a hysterectomy specimen), one placental site nodule (PSN), one case of placental site trophoblastic tumor (PSTT), and 4 choriocarcinomas.

Immunohistochemistry

Serial sections of 4–6 μm were cut from the paraffin blocks and mounted on APES-coated slides, deparaffinized in xylene, and rehydrated in graded alcohol to TBS (50 mM Tris, 150 mM NaCl, pH 7.4). The slides were microwaved for 4 × 5 minutes in 10 mM citrate, pH 6.0. After cooling down for 20 minutes, the slides were washed in TBS, blocked for 30 minutes at room temperature with normal serum (rabbit IgG, ABC Kit, Vector Laboratories, Burlingame, CA) and detected with DAB-substrate kit (Vectastain). Two isoforms of C/EBP were then reacted with biotin-labeled antirabbit immunoglobulin (IgG), incubated with preformed ABC-complex (Vectastain, Vector Laboratories, Burlingame, CA) and detected with DAB-substrate kit (Vectastain). The slides were counterstained with hemalaun and mounted with glycerine/gelatine. The staining intensity was then evaluated independently by two observers with a score given as follows: − = negative, + = positive, 1+ = weak staining, 2+ = moderate staining, and 3+ = strong staining.

RESULTS

The staining results for C/EBP-β are summarized in Table 1 including the percentage of marked cells and staining intensity (1+, 2+, 3+). Immunohistochemistry is shown in Figure 1A–F and Figure 2A–D. In the hydatidiform moles, C/EBP-β was strongly expressed in the nuclei of the villous ST and in the trophoblast proliferations on the villous surface while villous CT was negative (Fig. 1A–C; Table 1). We had one case of an invasive mole where the invasion site showed 2–3+ staining intensity in 80% of the cells (Fig. 1D). Other hydatidiform moles showed a positivity for C/EBP-β in 50% to 60% of the cells with a 1–2+ staining intensity in the implantation site. The PSN also showed a positive nuclear staining for C/EBP-β (Fig. 1E, F; Table 1). In the PSTT, positive expression could be seen in 60% of the cells (Fig. 2A, B; Table 1). In choriocarcinomas, a positive nuclear staining for C/EBP-β was also found (Fig. 2C, D; Table 1). In these cases, staining was more heterogeneous and only 30% to 40% of the cells were positive.

DISCUSSION

In this study, we investigated for the first time the expression pattern of the most important member of the C/EBP family, C/EBP-β, in different gestational trophoblastic lesions using immunohistochemistry on paraffin sections. The C/EBP family are proteins that consist of an N-terminal activation domain, a DNA-binding basic region, and a leucine-rich dimerization domain termed “leucine zipper” and which contains leucine repeats that intercalate with repeats of the dimer partner. Six members of this family, most of them intronless, have been described: C/EBP-α, -β, -δ, -e, -γ, and -ζ (7,8,10). For the members of the C/EBP family it is possible to dimerize with other members of their family as well as with transcription factors of the NF-kB, the AP-1, the retinoblastoma families, PR, etc. (11–13). This ability to form homo- or heterodimers is dependent on the abundance of the C/EBP types or isoforms in the cell and their binding to other regulatory proteins. All C/EBP dimers bind to the same DNA sequence within these target genes (7,14).

Two isoforms of C/EBP-β exist. One is a 32 kDa full-length protein termed LAP (liver-enriched transcriptional activating protein) and the other is a 20 kDa truncated form termed LIP (liver-enriched transcriptional inhibitory protein) which lacks the transactivation domain.

<table>
<thead>
<tr>
<th>Structure</th>
<th>C/EBP-β expression</th>
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<tbody>
<tr>
<td>1. Hydatidiform mole</td>
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<tr>
<td>Villous cytotrophoblast</td>
<td>−</td>
</tr>
<tr>
<td>Villous syncytiotrophoblast</td>
<td>+, &gt;80% cells, 3+ intensity</td>
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<tr>
<td>Trophoblast proliferation</td>
<td>+, &gt;80% cells, 3+ intensity</td>
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<td>on villous surface</td>
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<tr>
<td>Implantation site</td>
<td>+, 50% to 60% cells, 1–2+ intensity</td>
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<tr>
<td>Invasion site of invasive</td>
<td>+, 80% cells, 2–3+ intensity</td>
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<tr>
<td>mole</td>
<td></td>
</tr>
<tr>
<td>2. PSN</td>
<td>+, 30% cells, 2+ intensity</td>
</tr>
<tr>
<td>3. PSTT</td>
<td>+, 60% cells, 2–3+ intensity</td>
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<tr>
<td>4. Choriocarcinoma</td>
<td>+, 30% to 40% cells, 1–2+ intensity</td>
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CEBP, CCAAT/enhancer-binding protein, PSN, placental site nodule; PSTT, placental site trophoblastic tumor.

− = negative, + = positive, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining.
FIG. 1. Immunohistochemical localization of C/EBP-β in hydatidiform moles and placental site nodule. Expression of C/EBP-β in hydatidiform moles (A–D). Strong nuclear expression (brown color) in villous syncytiotrophoblast and in trophoblast proliferations on villous surface (A–C); villous cytotrophoblast is negative. Expression in the invasion site of one invasive mole (D). Immunohistochemical detection of C/EBP-β in a placental site nodule (E, F). Note positive nuclear staining for C/EBP-β with weaker expression intensity than in the hydatidiform moles.
The binding of the LAP isoform of C/EBP-β with the human aromatase cytochrome P450 gene (CYP19) in the human placenta has been previously described (15). In this study, we examined the expression pattern of C/EBP-β in different GTDs. GTDs are the result of pathological placental development and are associated with abnormal proliferation and/or invasion of trophoblast. One group of GTDs are neoplastic and include the incomplete, the complete, and the invasive hydatidiform moles, choriocarcinomas, PSTTs, and the epithelioid trophoblastic tumor (ETT). The second group are self-limited benign lesions that include the PSN and the exaggerated placental site (EPS) (16–19). Based on the morphological examination of the different subpopulations of the trophoblast, it is assumed that the PSN, the ETT, the EPS, and the PSTT are lesions of IT. The EPS and the PSTT develop from the implantation site IT and the PSN and the ETT derive from the chorionic-type IT (17,20,21).

One study showed that in the normal human placenta, there is strong nuclear C/EBP-β staining in villous ST and extravillous IT, whereas villous CT is negative (9). In our study, we found that in hydatidiform moles, C/EBP-β is highly expressed in the villous ST and in the trophoblast on the villous surface that is considered to be a mixture of CT, ST, and IT (16,21). Villous CT was negative. Also the implantation site showed a positive nuclear staining as did the invasion site in one case of an invasive mole. In addition, C/EBP-β expression could also be found in the PSN, the PSTT, and in the choriocarcinomas with a decreasing staining intensity from the PSTT to the choriocarcinomas. Our findings thus suggest that C/EBP-β can be potentially useful as an additional diagnostic marker in GTDs.
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REFERENCES


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