Co-localization of KLF6 and KLF4 with pregnancy-specific glycoproteins during human placenta development

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To cite this version:
Gene expression pattern

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Received 27 February 2001; received in revised form 17 April 2001; accepted 23 April 2001

Abstract

Pregnancy-specific glycoproteins (PSGs) are major placental proteins essential for the maintenance of normal gestation. However, little is known about their gene expression regulation during placentation. It was previously demonstrated that the human core promoter binding protein recently renamed Krüppel-like factor (KLF) 6 binds to a highly conserved sequence within the PSG promoters and is mainly expressed in human term placenta. Here, we determined the expression pattern of the 13 other KLFs during human placental development. We demonstrate that eight KLFs exhibit specific expression patterns in human placental tissues and membranes, in favor of a functional cooperation of specific KLFs during placentation. In addition, we demonstrate that KLF6, KLF4 and PSG proteins are co-expressed in same cell types of placental villi and membranes. This experimental evidence further strengthens the potential cross talk of both transcription factors for PSG gene regulation in vivo. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Development; Human placenta; Trophoblast; Krüppel-like factor; Pregnancy-specific glycoprotein

The placenta is composed by the extraembryonic cytotrophoblastic cells, which progressively differentiate into multinucleated syncytiotrophoblasts (Kaufmann and Burton, 1994). The syncytiotrophoblasts synthesize a number of pregnancy proteins such as the pregnancy-specific glycoproteins (PSGs) (Cross et al., 1994).

The PSGs are the major placental proteins secreted to maternal circulation during pregnancy with still unresolved function (Khan et al., 1992). PSG biosynthesis is mainly regulated at transcriptional level during human placental development (Bocco et al., 1989), implicating an in vitro interaction of GC-box motif with KLF6, which could be functional in vivo for regulation of PSG genes (Koritschoner et al., 1996, 1997; Slavin et al., 1999).

KLF6 belongs to a new family of Krüppel zinc finger transcription factors (Turner and Crossley, 1999). The KLF molecules have been shown to exhibit important tissue specific developmental regulatory functions (Dang et al., 2000). All KLFs could bind similar promoter sequences, present in several genes (Okano et al., 2000). Together, these observations are in agreement with cooperation between specific KLFs aimed to orchestrate tissue-specific developmental gene expression.

In order to gain knowledge regarding the involvement of KLFs during placentation, we analyzed the expression of the fourteen KLFs along with the PSG products during different steps of human pregnancy.

1. Results and discussion

KLF1/EKLF (Miller and Bieker 1993), KLF5/BTEB2/IKLF (Anderson et al., 1995), KLF9/BTEB (Imataka et al., 1992), KLF12/AP2-rep (Imhof et al., 1999), KLF13 and KLF14/KKLF (Scohy et al., 2000) were never expressed at detectable levels in placental tissues. In contrast, the eight other KLFs showed a differential expression pattern in placental tissues and/or membranes (Table 1).

Showing a similar expression pattern than PSG tran-
Table 1
Summary of the KLF gene expression profiles during human placentation in villi and placental membranes

<table>
<thead>
<tr>
<th></th>
<th>PSG-5</th>
<th>KLF2 (LKLF)</th>
<th>KLF3 (BKLF)</th>
<th>KLF4 (GKLF)</th>
<th>KLF6 (CPBP)</th>
<th>KLF7 (UKLF)</th>
<th>KLF8 (BKLF3)</th>
<th>KLF10 (TIEG1)</th>
<th>KLF11 (TIEG2)</th>
<th>KLF12 (AP-2rep)</th>
<th>KLF1, -5, -9, -13, -14</th>
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<tbody>
<tr>
<td>Placental tissues</td>
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<td>6th week (first trimester)</td>
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<td>14th week (second trimester)</td>
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<td>29th week (third trimester)</td>
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<td>37th week (term)</td>
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<td>Placental membranes</td>
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<td>Trophoblastic cells</td>
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<td>Primary culture of trophoblasts</td>
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<td>JEG-3</td>
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scripts (Streydio and Vassart, 1990), four KLF members were expressed at all stages analyzed by reverse transcriptase–polymerase chain reaction (RT–PCR). As expected, the KLF6 gene expression correlated directly with the PSG pattern, further supporting its function as regulator of PSG genes transcription in vivo.

The function assigned for the KLF2/LKLF molecule is also of biological interest for placental development. This molecule is involved in the assembly of the vascular tunica and blood vessel stabilization during development (Kuo et al., 1997), both of which are biological functions also necessary for placental vasculogenesis (Cross et al., 1994).

KLF11, also called TIEG-2, was initially identified as a TGFβ-inducible immediate-early gene transcription factor (Cook et al., 1998). Taking into account that the TGFβ pathway plays an essential role for normal placentation, it is interesting to propose that KLF11 also mediates TGFβ signaling in this tissue. Additionally, it was demonstrated that KLF6 is also involved in the TGFβ signaling pathway (Kim et al., 1998; Kojima et al., 2000).

Four KLF family members were not detected during the first early steps of pregnancy, namely KLF3/BKLF (Crossley et al., 1996), KLF4/GKLF (Shields et al., 1996), KLF7/UKLF (Matsumoto et al., 1998) and KLF10/TIEG1 (Cook et al. 1998). Nevertheless, the KLF4 expression became detectable and was co-expressed together with KLF6 in the placenta from mid-gestation until parturition. Note that these proteins co activated the human keratin-4 promoter (Okano et al., 2000).

At term, human placenta expressed eight KLFs, with similar expression whether the placenta was obtained by chirurgical cesarean or spontaneous parturition, excluding implications of these KLFs for labor parturition.

As previously described (Chou and Zilberstein, 1990), PSG is expressed in placental villi (Fig. 1C,I). Interestingly, KLF6 was also detected in placental villi (Fig. 1D,F), but was...
mainly co-expressed with PSG in syncytiotrophoblastic cells (Fig. 1J). The same expression pattern was found for KLF4 (Fig. 1E,G,K), strongly supporting the potential involvement of this molecule in concert with KLF6 for the in vivo PSG gene regulation. This hypothesis is further strengthened by the presence of consensus sequence for KLF4/GKLF binding in PSG promoter (Panzetta-Dutari et al., 2000). The KLF6, KLF4 and PSG co-localization was still observed on primary cultures of cytotrophoblasts cells (Fig. 1N±P).

We also analyzed KLF expression in the placental membranes at term where PSG biosynthesis was also reported (Plouzek and Chou, 1991). Expression of PSG mRNA was confirmed in both, chorionic and amniotic membranes along with seven KLFs transcripts. Interestingly, the amnion and chorion membranes, which are from embryonic and extra-embryonic origin, respectively, showed differences in the KLF expression pattern. The amnion and chorion membranes have been implicated in erythropoiesis during pregnancy (Moritz et al., 1997). It is interesting to mention that both KLF2 and 3 share regulatory roles during erythropoiesis mainly through the control of globin gene expression (Dang et al., 2000).

KLF6, KLF4 and PSG molecules are also co-expressed in amniotic (Fig. 2C,D,F,G,H and data not shown) as well as in chorionic membranes (Fig. 2K,L and data not shown) at mRNAs and protein levels.

In conclusion, we demonstrate that human KLFs are specifically expressed in placenta throughout pregnancy. The KLF6 and KLF4 are co-expressed with PSG molecules in placenta as well as in villi and membranes stressing their cooperation for PSG gene expression control.

2. Methods

In situ hybridization was performed as already described (Slavin et al. 1999). Primary culture of cytotrophoblasts was realized according to Jacquemin et al. (1998) and characterized using a specific marker, the cytokeratin 7 (Blaschitz et al., 2000). The template cDNAs used to generate PSG,
GKLF and CPBP digoxigenin-labeled riboprobes (DIG RNA labeling kit SP6/T7, Roche) have been described in Shields et al. (1996) and Koritschoner et al. (1997). The immunohistochemistry experiments were performed as previously described (Sapin et al., 1997) using antibodies against GKLF (M-19), KLF6/ZIP (R-173, Santa Cruz Biotechnology), PLH (Sigma) and PSG (Dako).

Acknowledgements

We thank the Centre Hospitalo-Universitaire for its financial support (PHRC 2000). L.B. is supported by a MERT grant.

References


