The immunolocalization of Bcl-2 in human term placenta

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Summary

Purpose: To study the immunolocalization of the Bcl-2 protein in formalin-fixed placental tissue collected from uncomplicated term pregnancies.

Methods: A total of 19 human term placentas of 38-41 weeks' gestation, 11 obtained from spontaneous deliveries and eight from elective caesarean sections prior to labour were included. Sections were incubated with an antibody to the Bcl-2 protein and light microscopy was used to evaluate Bcl-2 staining.

Results: The anti-apoptotic Bcl-2 protein was expressed diffusely throughout the cytoplasm of the syncytiotrophoblast with much less intensive staining in cytotrophoblast and mesenchymal cells. Bcl-2 expression was reduced or lost in areas of syncytial sprouts. No differences in Bcl-2 staining were observed between placentas obtained after spontaneous deliveries and those collected before the onset of labour after elective caesarean section.

Conclusion: Expression of the anti-apoptotic Bcl-2 protein in human term placenta does not seem to be influenced by parturition. Additionally, Bcl-2 expression might be an important factor in the regulation of apoptosis in the human trophoblast and thus in maintaining placental function during gestation.

Key words: Bcl-2 expression; Placenta; Immunohistochemistry.

Introduction

The Bcl-2 proto-oncogene encodes a 26-kd protein on chromosome 18, localized to mitochondrial and perinuclear membranes, endoplasmatic reticulum and the nucleus [1, 2]. Bcl-2 is believed to be involved in tumorigenesis and in the regulation of programmed cell death (apoptosis) [3, 4]. Apoptosis is the process of programmed cell death, distinct from necrosis, that plays an essential role in cell turnover and tissue homeostasis, embryogenesis, cytotoxic immunological reactions, development of the nervous system and endocrine-dependent tissue atrophy. External or internal signals can promote apoptosis [5, 6].

The biochemical hallmark of apoptosis is the degradation of the genomic DNA, an irreversible event committing the cell to die. Morphological features of the final stages of apoptosis include chromatin aggregation, nuclear and cytoplasmic condensation, partition of cytoplasm and nucleus into membrane bound vesicles (apoptotic bodies) which contain ribosomes, mitochondria and nuclear material [5-9].

The apoptotic process is supposed to be regulated by several pro- and anti-apoptotic oncogenes and tumour-suppressor genes as p53, Bax, Bak and Bcl-2 [10-16]. The Bcl-2 gene was first discovered in follicular lymphomas possessing a t(14; 18) (q32, Q21) translocation and is associated with the prevention of apoptosis whereas Bax, Bak and p53 are thought to act as programmed cell death promoters [10-16].

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The human placenta is the central organ of the fetal-placental-maternal unit and plays a fundamental role in the nutritient and gas transfer to the developing embryo and fetus. Placental malfunction may lead, e.g., to fetal growth restriction or miscarriage. In early pregnancy the cytotrophoblast rapidly proliferates but with advancing gestation the trophoblast shows evidence of maturation and differentiation. The syncytiotrophoblast is formed from fused villous trophoblast cells and constitutes the narrow epithelial layer separating maternal from fetal blood [17]. Bcl-2 expression has been previously described in human placenta [18-24]. Decreased expression of Bcl-2 along with terminal differentiation and maturation in late gestation compared to first trimester placentas was noted [18]. Furthermore, in complete hydatidiform mole, in choriocarcinoma and in placental tissue of miscarriages weaker expression of Bcl-2 protein compared to healthy early gestation was observed [10, 19, 23].

We wanted to determine the pattern of expression and the immunolocalization of the Bcl-2 proto-oncogene in placentas of healthy term pregnancies before the onset of labour and after spontaenous delivery.

Materials and Methods

Tissue samples

The study presented was carried out in the Department of Obstetrics and Gynecology at the University of the Saarland, Germany. For immunohistochemistry, 19 placentas from uncomplicated term pregnancies of 38-41 weeks' gestation were obtained immediately after labour and vaginal delivery or after elective caesarean section under epidural anaesthesia without prior labour. Two random biopsies of each placenta were collected. The tissue was free of visible infarcts, calcifi-

cations or hematoma. All women were normotensive, had no medical illness, did not take any medication and denied smoking and alcohol use during pregnancy. Placentas from pregnancies with intrauterine growth restriction, premature rupture of membranes or clinical or histological signs of chorioamnionitis were excluded from the study.

Immunohistochemistry

Immunohistochemical studies were performed on formalin-fixed (3.7% buffered formaldehyde), paraffin-embedded tissue; 5 μm-thick placental sections were mounted on acid-cleaned microscope slides pretreated with 0.01% aqueous solution of poly-L-Iysine. Deparaffinization and rehydration were performed through xylene and a graded, descending series of ethanol and distilled water.

Afterwards deparaffinization sections were then incubated in 30% H_2O_2 in absolute methanol for 10 min to inactivate endogenous peroxidase. Then slides were rinsed three times in 0.05 mol/l tris-buffered saline (TBS), pH 7.6. The primary antibody was diluted in TBS, pH 7.6. Then the sections were incubated with the primary mouse monoclonal Bcl-2 antibody (1:50) for 24 h at a temperature of 4°C. The primary monoclonal mouse Bcl-2 antibody was purchased from Oncogene Science, Inc., MA, USA. After being washed 3 times with TBS, slides were incubated with biotinylated F(ab')2 fragment of rabbit anti-mouse immunoglobulins (1:200; Dako Diagnostika, Hamburg, Germany) for 20 min at room temperature. They were further washed three times with TBS followed by incubation with peroxidase-labelled streptavidin solution (streptAB-complex/HRP, 1:300; Dako Diagnostika, Hamburg, Germany) for 20 min at room temperature. The slides were washed again three times with TBS, pH 7.6. The streptavidin-biotin complex was visualized with DAB (3,3 diaminobenzidine tetrahydrochloride; Dako Diagnostika, Hamburg, Germany) by incubation for 10 min. Finally, the sections were washed in distilled water, counterstained with hematoxylin, dehydrated and mounted in xylene.

Negative controls were performed on a section of each sample by replacement of the primary antibody with mouse immunoglobulin in the same concentration as the primary antibody with all other steps unchanged. Further controls omitting the secondary antibody were also performed. Controls were always negative.

Data analysis

Two sections for each placental biopsy were examined and the results are given as mean values. Slides were evaluated by two independent observers with an Olympus BH2 microscope. Cytoplasmatic staining was the criterion for a positive Bcl-2 staining. The immunohistochemical staining for Bcl-2 was recorded as ++ if intense staining was seen, + and ± were regarded as moderate or trace negative staining intensity and – was evaluated as absent staining. The percentage of positively stained tissue was scored semiquantitatively on a scale with three grades: – no positive staining; 1, <25% positively stained; 2, 26-50% positively stained; 3, >51% positively stained.

Results

Bcl-2 immunoreactivity was observed in all tissue sections with a diffuse expression pattern. Bcl-2 expression was confined to the cytoplasm of the syncytiotrophoblast of intermediate and terminal villi in all examined sections, whereas the underlying cytotrophoblast layer as well as the various cellular components of the villous stroma showed absent or very modest immunostaining in comparison to the syncytiotrophoblast layer. In only two cases did the villous cytotrophoblast show focal (<25%) and discrete Bcl-2 immunoreactivity. In all other cases the villous cytotrophoblast was negative for Bcl-2. The percentage of immunoreactive tissue and the intensity of staining are summarized in Table 1.

In general, we observed moderate to intensive staining in the cytoplasm of the syncytiotrophoblast, with >51% of the syncytium showing immunoreactivity in almost all tissue sections. Very few terminal villi (<25%) of the syncytiotrophoblast were stained with very modest intensity.

In the chorionic plate as well as in the basal plate we did not observe Bcl-2 expression in extravillous cytotrophoblastic cells nor in connective tissue cells nor in decidual cells of the tissue sections used in this study.

Decreased Bcl-2 immunoreactivity was observed in areas of syncytial sprouts compared to the immunostaining in the adjacent syncytiotrophoblast in some cases. Furthermore clearly necrotic areas were negative for Bcl-2.

We could not detect any differences in Bcl-2 expression between placental tissue obtained after spontaneous vaginal delivery and those placentas obtained after elective caesarean section prior to labour.

Table 1. — *Results of Bcl-2 immunohistochemistry, expressed as intensity and extent of immunostaining, in uncomplicated term placentas*

			Bcl-2 staining			
			CT		ST	
Case No.	G.A. (weeks)	Mode of delivery	Intensity	Extent	Intensity	Extent
1	38.1	CS	–	–	++	3
2	38.2	VD	–	–	++	3
3	38.2	CS	–	–	+	3
4	38.3	VD	–	–	+	3
5	38.4	VD	–	–	+	3
6	38.5	CS	±	1	++	3
7	38.6	CS	–	–	+	3
8	39.0	VD	–	–	++	3
9	39.1	VD	–	–	+	3
10	39.1	VD	–	–	++	3
11	39.3	CS	–	–	+	3
12	39.4	CS	–	–	±	1
13	39.4	VD	–	–	++	3
14	39.6	VD	±	1	±	1
15	40.1	CS	–	–	+	2
16	40.2	VD	–	–	++	3
17	40.4	VD	–	–	+	3
18	40.6	VD	–	–	±	1
19	41.0	CS	–	–	+	2

Bcl-2 staining intensity: ++: intense; +: moderate; ±: trace; –: negative % of positive Bcl-2 staining: –: absent; 1: <25%; 2: 26-50%; 3, >51%.
CS: elective caesarean delivery prior to labour; VD: spontaneous vaginal delivery; GA: gestational age.

Fig. 1

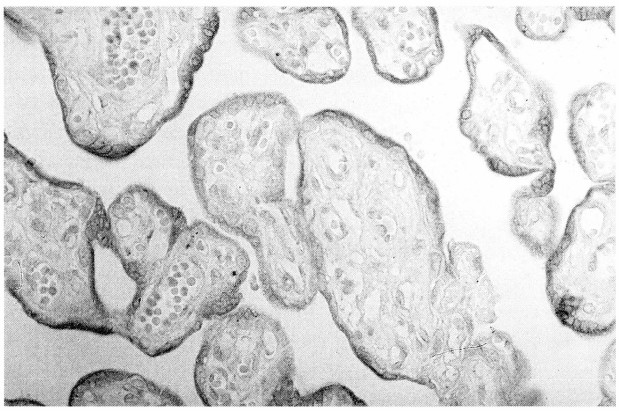
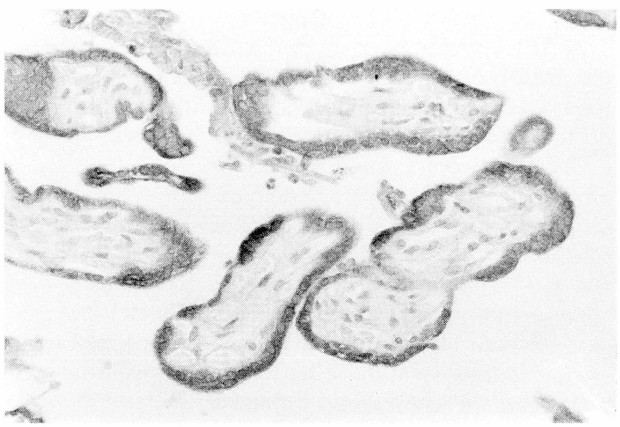


Fig. 2



Figures 1, 2. — Diffuse, moderate (Figure 1) to intensive (Figure 2) Bcl-2 immunoreactivity confined to the syncytiotrophoblast layer. Villous cytotrophoblast and villous stroma is negative for Bcl-2, original magnification x 250.

Discussion

In the present study we have investigated the presence of the protein product of the proto-oncogene Bcl-2 in uncomplicated human term (38-41 weeks) gestations. Data presented here illustrate that Bcl-2 protooncogene product is expressed in a diffuse manner in the human term placenta with the protein being predominantly present within the syncytiotrophoblast layer and to a far lower extent in the underlining villous cytotrophoblast. Decidual cells, stromal tissue and the extravillous cytotrophoblast did not show Bcl-2 immunoreactivity. Furthermore, no differences in Bcl-2 expression between placental tissue obtained from spontaneous vaginal deliveries and elective caesarean sections prior to labour could be noted.

The human placenta is the central organ of the fetal-placental-maternal unit and plays a fundamental role in the nutritient and gas transfer to the developing embryo and fetus. Placental malfunction may lead, e.g., to fetal growth restriction or miscarriage [25, 26].

In the human placenta cell development and function depends on the balance among proliferation of cells, maturation and cell death. Cell death can occur by either of two distinct mechanisms, necrosis or apoptosis [17]. Trophoblast apoptosis in term placental villi occurring with low incidence has been previously reported by our group and by others [27-30].

The syncytiotrophoblast layer is the terminally differentiated, multinucleated syncytium of the villous tree without any proliferative capacity and it separates maternal from fetal blood flow [17, 30]. This epithelial layer is formed from fused villous cytotrophoblast and is essential to preserve normal maternal-fetal transport, secretory as well as immunological functions of the villous tree throughout gestation [17, 30]. Therefore studies on trophoblast homeostasis and regulation of apoptosis within the placental villi are of actual interest.

There are a number of oncogenes that regulate apoptosis. An important member of these oncogenes are the Bcl-associated genes [10, 11]. One member of the Bcl-family is Bcl-2 which is known to inhibit apoptosis. Proto-oncogene Bcl-2 prolongs the survival of certain types of cells and is expressed in proliferating cells in which apoptosis needs to be avoided [10-17]. Besides its role in tumorigenesis, its physiological expression has also been demonstrated in various human tissues characterized by apoptotic cell death, including hormonally regulated glandular epithelium such as skin and intestine and long-living postmitotic cells as neurons [12]. It is regarded as an essential feature of normal physiology during mammalian embryogenesis by regulating cell death during development [3, 4].

It was postulated that there is a differentiation-dependent pattern of Bcl-2 expression in human placenta with decreased expression along with terminal differentiation and maturation of the trophoblast in late gestation compared to early gestation [10, 18-20]. Bcl-2 expression was also supposed to be associated mainly with activated and undifferentiated cells which are undergoing terminal differentiation and need protection from apoptosis [1, 2, 19, 20]. Furthermore, Bcl-2 expression in the syncytiotrophoblast of failing pregnancies, choriocarcinoma and tissue of complete hydatidiform mole was shown to be decreased in contrast to the apoptotic index, which was increased in pathologic gestation [10, 19, 23]. In further studies an inverse relationship between Bcl-2 expression and p53 expression as well as Bax expression was found in the gestational trophoblast of a complete hydatidiform mole [24].

Our data, as reported in the results section, are in agreement with recent previous reports dealing with the expression of the anti-apoptotic Bcl-2 protein in human term placenta [10, 18-22]. Several authors have reported on Bcl-2 staining in the syncytiotrophoblast layer with lesser staining of the underlying cytotrophoblast in human term placentas [10, 18-22]. Thus, expression of the anti-apoptotic Bcl-2 protein in the syncytiotrophoblast might be involved in the preservation of syncytiotrophoblast function until delivery.

In our study Bcl-2 protein was not expressed in the villous cytotrophoblast, except in two cases with very distinct immunostaining, nor in the extravillous trophoblast,

nor in decidual cells, which is in agreement with results reported by others [22, 24]. Furthermore, our results like those of Cirelli and co-workers indicate, that Bcl-2 expression does not seem to be related to labour [31].

The final consequence of apoptosis is nuclear shrinkage and extrusion of groups of apoptotic nuclei together with surrounding cytoplasm, forming the so-called syncytial sprouts. In some cases in our study, formation of syncytial sprouts within the syncytiotrophoblast layer was associated with decreased syncytiotrophoblastic Bcl-2 expression. This fact has been also described by Kim and co-workers [18].

Although the number of samples studied was small, and we have not examined further apoptosis-related proteins yet, our previous results and this study suggest, that Bcl-2 expression within the syncytiotrophoblast layer may play a role in the preservation of placental function during late gestation, which does not seem to be related to labour.

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