www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2026;15(1):e150115
doi: 10.7363/150115
Received: 2025 Apr 28; revised: 2025 Jul 03; rerevised: 2026 Mar 29; accepted: 2026 Mar 31; published online: 2026 Apr 30

Original article

Homeodomain-only protein homeobox (HOPX) expression in the human placenta: a new actor in maternal fetal tolerance during gestation?

Gavino Faa[1], Monica Piras[1], Tiziana Cabras[2], Barbara Manconi[2], Alessandra Olianas[2], Flaviana Cau[3], Marco Piludu[4], Federica Iavarone[5,6], Irene Messana[2], Cristina Contini[2], Massimo Castagnola[7]

[1]Department of Medical Sciences and Public Health, University of Cagliari, Monserrato, Italy
[2]Department of Life and Environmental Sciences, University of Cagliari, Monserrato, Italy
[3]Clinical Pathology Department, Ospedale N. Signora di Bonaria, San Gavino, Italy
[4]Department of Biomedical Sciences, University of Cagliari, Monserrato, Italy
[5]Department of Biotechnological Sciences, Catholic University, Rome, Italy
[6]IRCCS Foundation University Hospital A. Gemelli, Catholic University, Rome, Italy
[7]European Research Center on Neuroscience, IRCCS Santa Lucia, Rome, Italy

Abstract

HOPX (homeodomain-only protein), a member of the homeodomain family, is involved in various developmental processes and immune regulation. This study highlights HOPX expression in human placenta and its potential role in modulating maternal immune responses during pregnancy. Twenty-four human placental samples, ranging from 11 to 40 weeks of gestation, were analyzed using immunohistochemistry to analyze HOPX expression. The results showed that HOPX was expressed predominantly in the syncytiotrophoblasts of placental villi, with heterogeneus distribution of immunoreactivity. In some placentas, HOPX was uniformly expressed across villi, while in others expression was absent. No significant expression of HOPX was observed in cytotrophoblasts, Hofbauer cells, or developing vascular structures. HOPX may play a key role in promoting maternal immune tolerance by modulating the activity of maternal T-cells in the placental interface. The variability in HOPX expression could indicate its involvement in pathological conditions, such as maternal non-acceptance of the fetus, and may be linked to pregnancy complications. Furthermore, the presence of HOPX in the nuclei of syncytiotrophoblasts underscores its potential role as a transcription factor involved in placental development and immune regulation. This study provides novel insights into the role of HOPX in maintaining maternal-fetal immune tolerance and highlights the need for further investigation into its

involvement in pregnancy-related immune responses and complications.

Keywords

HOPX, gestation, T-lymphocytes, placentas.

Corresponding author

Monica Piras, Department of Medical Sciences and Public Health, University of Cagliari, Italy; ORCID: 0000-0002-1068-1781; tel.: +39 3495846842; e-mail: monica.piras@unica.it.

How to cite

Faa G, Piras M, Cabras T, Manconi B, Olianas A, Cau F, Piludu M, Iavarone F, Messana I, Contini C, Castagnola M. Homeodomain-only protein homeobox (HOPX) expression in the human placenta: a new actor in maternal fetal tolerance during gestation? J Pediatr Neonat Individual Med. 2026;15(1):e150115. doi: 10.7363/150115.

Introduction

The homeodomain-only protein (HOPX, Swiss-Prot code Q9BPY8) is a member of the homeodomain family which plays a key role in physiology, particularly during the development of human embryos, through linking extracellular signals to gene regulatory programs, being able to regulate a wide array of genes [1]. Homeobox genes are a family of regulatory genes sharing a highly conserved 183-nucleotide homeobox sequence, and coding for nuclear proteins, termed the homeodomain, that act as transcription factors [2]. Multiple homeobox gene families have been identified, including *MSX*, *PAX*, *EMX* and *Hox* [3]. The proteins encoded by homeobox genes are involved in embryogenesis, organogenesis, as well as in carcinogenesis [4]. The human *HOPX* gene is located on chromosome 4 and consists of seven exons. HOPX protein, the smallest known homeodomain-containing protein, is composed of 73 amino acids. The 3D structure revealed that HOPX forms three alpha helices which fold into a helix-turn-helix motif characteristic of the homeobox. Moreover, HOPX contains a homeodomain motif that acts as an adapter protein to mediate transcription [5]. HOPX is also implicated in the differentiation of keratinocytes in interfollicular epidermis in mammalian skin.

During embryo development, HOPX is involved in embryogenesis of multiple organs and tissues, including the heart and the central nervous system [6]. In the immune system, HOPX is expressed in Th1 cells, protecting them against apoptosis, and in peripheral Treg cells, in which HOPX blocks interleukin-2 (IL-2) production [7]. Furthermore, HOPX is expressed in CD4+ and CD8+ T-lymphocytes, in B-lymphocytes, and in most natural killer (NK) and natural killer T (NKT) cells [8]. Recently, HOPX has been reported to act as a negative regulator of T-lymphocytes, inhibiting their proliferation and activation [9].

Since the mechanisms underlying maternal acceptance of the fetus, who expresses paternally inherited alloantigen, have not yet been fully clarified [10], the aim of this work was to analyze the expression of HOPX in the human placenta, in order to verify if HOPX expression in placental cells could play a role in down-regulating the activation of maternal T cell and their aggressiveness against the embryo and fetus during gestation.

Materials and methods

The study protocol for the use of human subjects in research was approved by the Human Research Ethics Committee of the Medical School, University of Cagliari, Italy (Prot. 13/C.E./05). The samples of 24 human placentas, ranging in gestational age from 11 up to 40 weeks, were investigated in this study. Each sample was fixed in 10% buffered formalin for at least 20 h, routinely processed, paraffin-embedded; 4 µ-thick sections were cut for morphological study, hematoxylin and eosin staining. Subsequently, sections were deparaffinized in xylene, rehydrated in a graded alcohol series and then equilibrated in PBS. Antigen retrieval was performed by citrate buffer (0.01 M; pH 6) at 95ºC. Prior to incubation in the primary antibody, endogenous peroxidase activity was quenched with a solution of hydrogen peroxide 3% in 100% methanol for 20 min. Then, the sections were incubated for 40 min with 5% skim milk solution to block non-specific sites. Sections were incubated over night at 4ºC in rabbit primary antiserum polyclonal antibody HOPX (Santa Cruz sc-30216 [rabbit polyclonal 1:200]) and 30 min in anti-rabbit peroxidase conjugated antibody (1:100, Chemicon International, Billerica, MA, USA) as secondary antiserum. 3,3'-diaminobenzidine (DAB) was used as final chromogen. For negative controls, sections were incubated in parallel with the antibody diluent alone. The antibody used has been validated by the manufacturer, confirming its specificity and expected staining pattern. Therefore, no additional positive control was included in this experiment. Sections were counterstained with Meyer's hematoxylin (Sigma), dehydrated through graded ethanols and xylene, and embedded in Vitro-Clud (Langenbrink, Emmendingen, Germany). The

sections were examined and photographed with a Leica microscope (Leica Microsystems, Bensheim, Germany). As positive control tissue for validating HOPX immunostain, samples of normal colorectal mucosa were utilized, being characterized by strong expression of HOPX [11]. A grading score was defined for a semiquantitative evaluation of HOPX immunoreactivity: 0 = no reactivity; 1 = focal immunoreactivity in < 10% of villi; 2 = immunostaining in > 10% and < 50% of villi; 3 = diffuse reactivity in > 50% of placental villi; 4 = diffuse immunoreactivity in terminal villi associated with immunoreactive extracellular vesicles in the intervillar spaces.

Results

Immunoreactivity for HOPX was observed in all placental samples analyzed in this study (see **Tab. 1**). Different degrees of immunostaining were observed, according with a grading system developed for the semi-quantitative evaluation of HOPX expression: 13 placentas showed diffuse immunostaining of syncytiotrophoblasts (grade 3), 5 were characterized by immunoreactivity in less than 50% of terminal villi (grade 2), with HOPX expression in 10-50% of villi, and 3 were characterized by a focal reactivity for HOPX in less than 10% of villi (grade 1). Furthermore, 3 cases showed a diffuse reactivity in villi associated with the presence of HOPX-reactive extracellular vesicles in the intervillous spaces (grade 4). No correlation was observed between HOPX expression and the gestational age. In placentas with grade 3 immunoreactivity, HOPX was expressed on the surface of most villi. Immunostaining of the surface syncytiotrophoblasts contrasted with the absence of any reactivity in the other cells of the placental villi, including cytotrophoblasts, and Hofbauer cells (**Fig. 1**). In cases with grade 2 immunoreactivity, we observed an uneven distribution of HOPX immunoreactivity (**Fig. 2**). In these cases, a strong immunoreactivity for HOPX on the surface of some villi contrasted with the absence of any significant expression in adjacent villi. A patchy distribution of HOPX immunostaining was detected in cases with grade 1 immunostaining, with few isolated immunoreactive villi surrounded by most placental villi devoid of any expression of HOPX (**Fig. 3**). At higher magnification, the expression of HOPX appeared restricted to the syncytiotrophoblasts, being mainly observed in the cytoplasm. We did not find a significant immunostaining in the other compartments of the villi, including cytotrophoblasts, Hofbauer cells and developing vascular structures (**Fig. 4**). At high power, we succeeded in having more information on the distribution of HOPX. In **Fig. 5** we show the restriction of reactivity for HOPX to the syncytiotrophoblasts covering the surface of placental villi showing reactivity of the entire cytoplasm and cell membrane. Moreover, strong immunostaining for HOPX was also observed in many nuclei of syncytiotrophoblast cells, which contrasted with the absence of any expression inside the nuclei of cytotrophoblasts. At high power, a mild reactivity for HOPX was also detected in the endothelium of the small developing intravillous vessels. The absence of HOPX expression in Hofbauer cells was confirmed at high power. Adjacent villi occasionally showed different degrees of immunoreactivity for HOPX, regarding both cytoplasmic and nuclear staining (**Fig. 6**). Immature trophoblasts did not show any reactivity for HOPX (**Fig. 7**). No significant expression of HOPX was detected in the inter-villi space in 16 out of 24 placentas. In the remaining 8 placentas, extracellular vesicles strongly immunoreactive for HOPX were observed in the intervillous spaces. In 1 case (see **Tab. 1**), HOPX-reactive extracellular vesicles were diffuse, representing the major site of HOPX expression in this placenta (**Fig. 8**).

Table 1. Grading of immunohistochemical reactivity for HOPX in human placentas.

Case	Week of gestation	HOPX grading [a]	Extracellular vesicles [b]
1	35	3	+
2	35	3	+
3	39	3	-
4	32	4	+ + +
5	20	1	+
6	24	1	+
7	13	3	-
8	11	3	-
9	11	2	-
10	12	2	-
11	12	3	-
12	11	1	-
13	13	2	-
14	20	3	-
15	21	3	-
16	24	3	-
17	40	4	+
18	39	2	-
19	22	3	+
20	37	3	-
21	28	2	-
22	33	4	+
23	12	3	-
24	40	3	-

[a] HOPX grading: 0 = no reactivity; 1 = focal immunoreactivity in < 10% of villi; 2 = immunostaining in > 10% and < 50% of villi; 3 = diffuse reactivity in > 50% of placental villi; 4 = diffuse immunoreactivity in terminal villi associated with immunoreactive extracellular vesicles in the intervillar spaces. [b] Extracellular vesicles: + = extracellular vesicles strongly immunoreactive for HOPX were observed in the intervillous spaces; +++ = HOPX-reactive extracellular vesicles were diffuse, representing the major site of HOPX expression in this placenta.

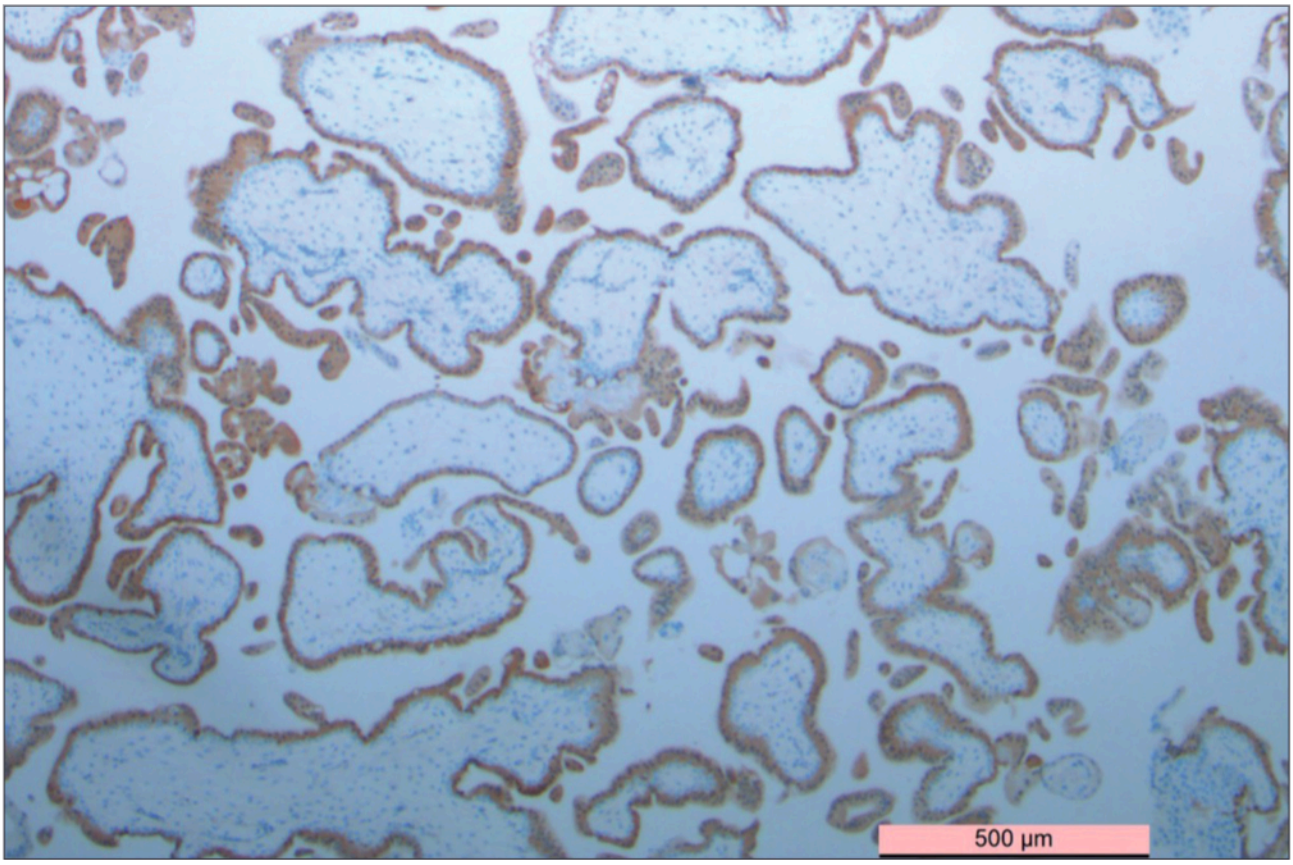

Figure 1. Evident immunolabeling for HOPX of the surface syncytiotrophoblasts.

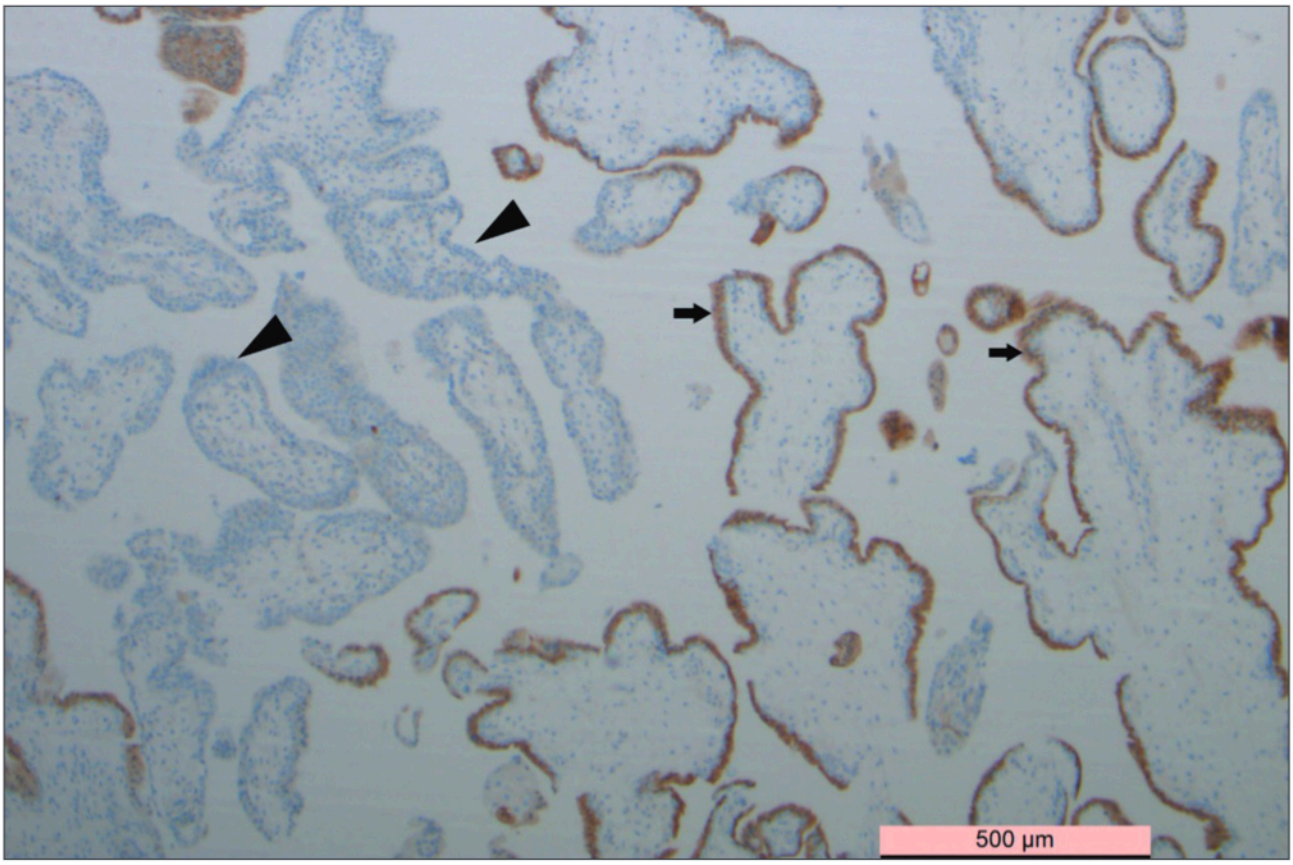

Figure 2. Strong immunolabeling for HOPX on the surface of some villi (arrows); significant absence of immunolabeling in other villi (arrowheads).

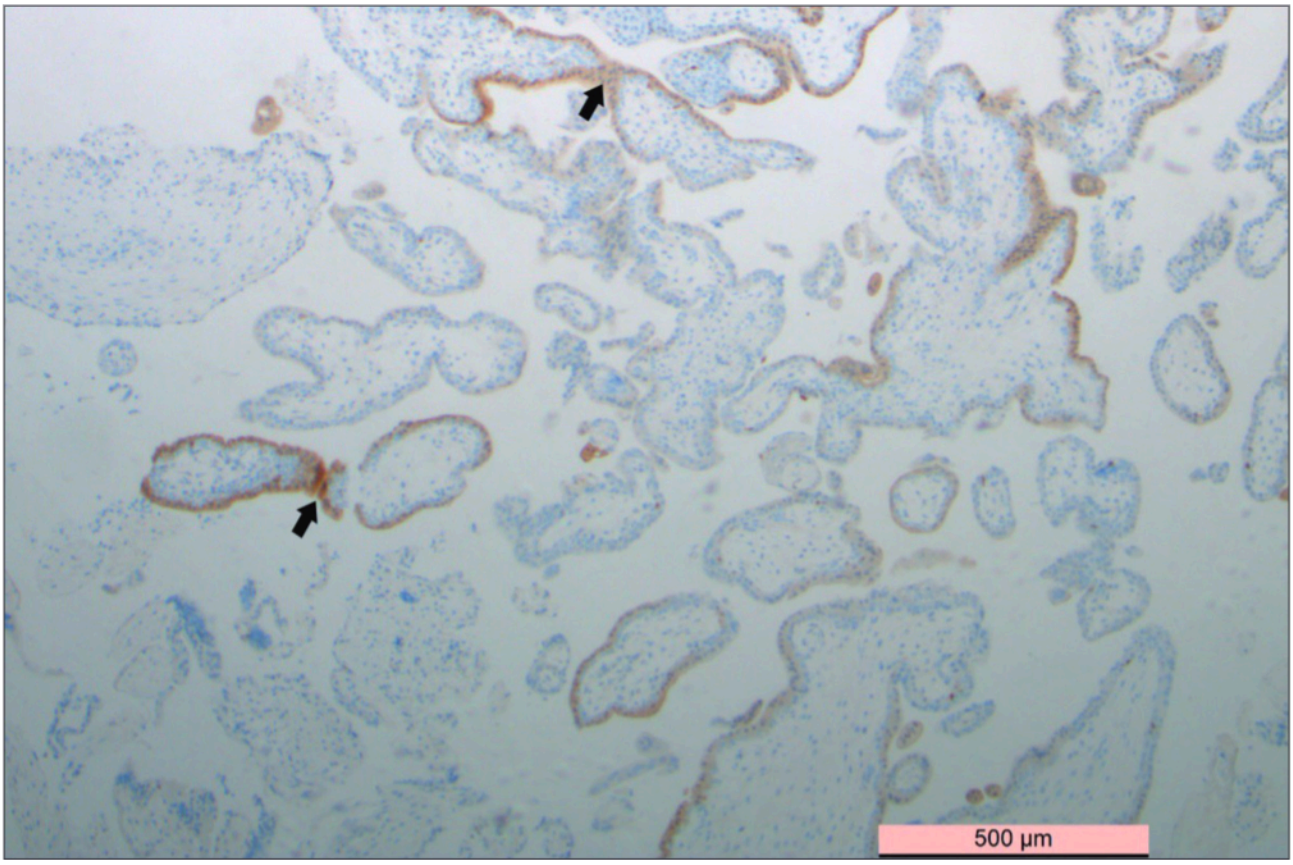

Figure 3. Reduced immunolabeling for HOPX, restricted to only a few villi (arrows).

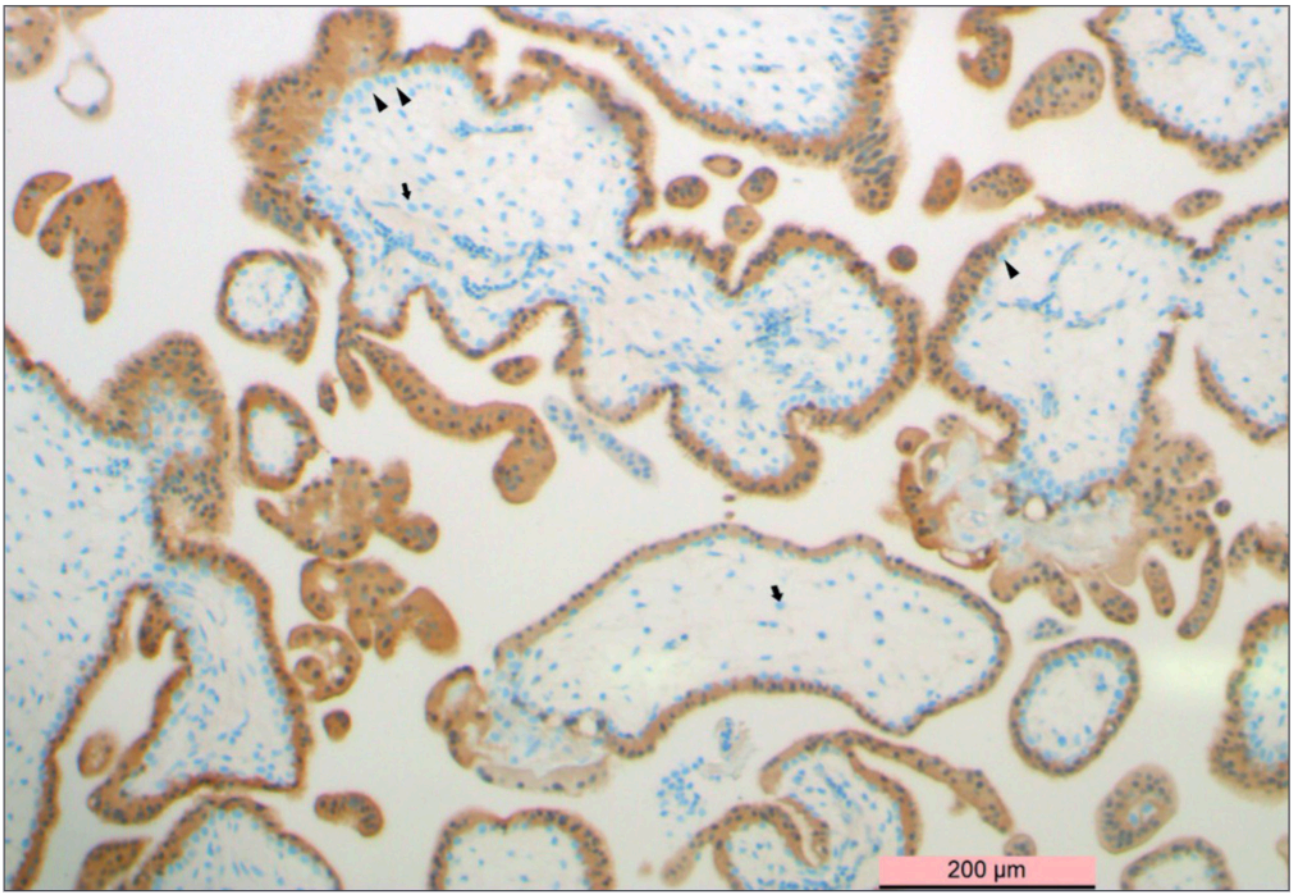

Figure 4. Absence of immunolabeling for HOPX in cytotrophoblast cells (arrowheads) and in Hofbauer cells (arrows).

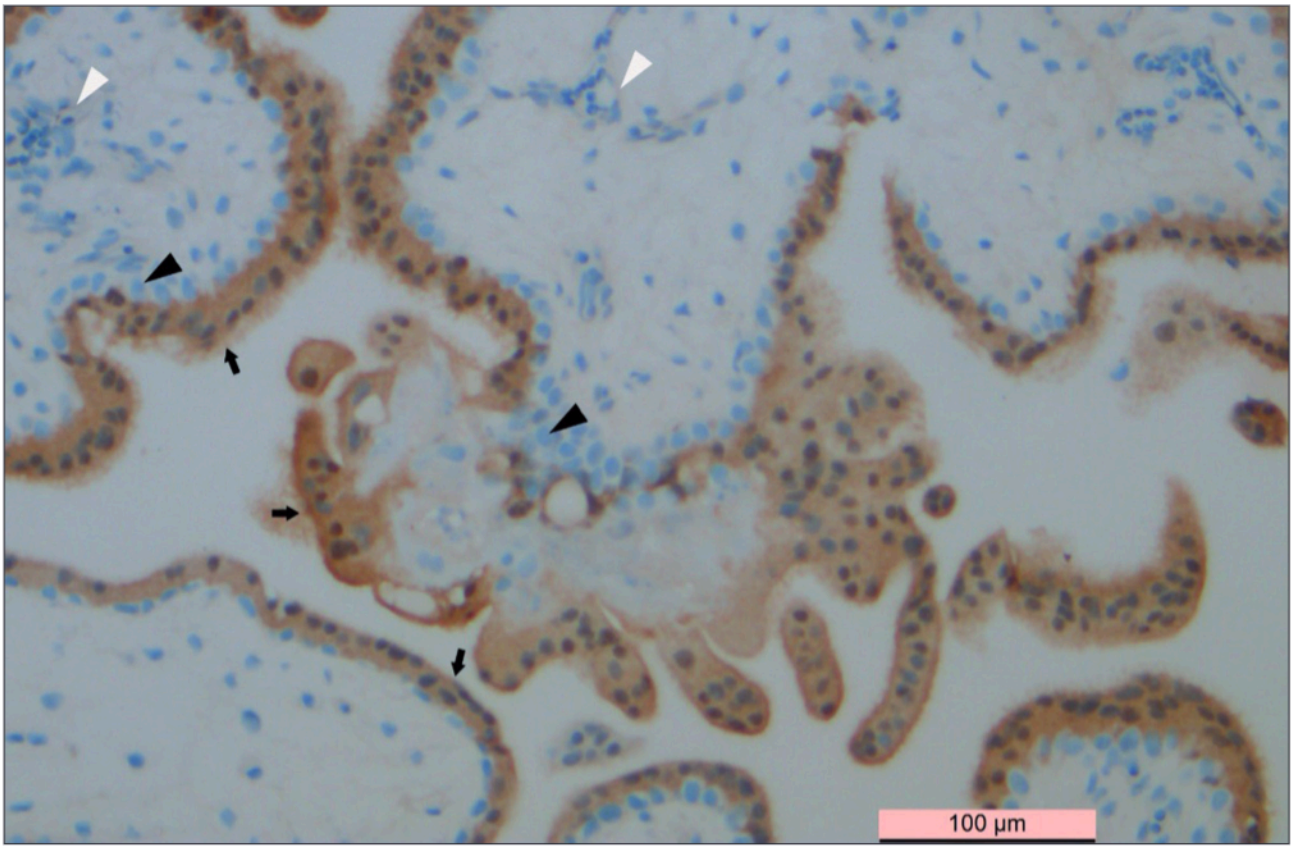

Figure 5. Strong immunolabeling for HOPX is observed in many nuclei of syncytiotrophoblast cells (arrows); absence of any expression inside the nuclei of cytotrophoblasts (arrowheads). A mild reactivity for HOPX is present in the endothelium of the small developing intravillous vessels (open arrow).

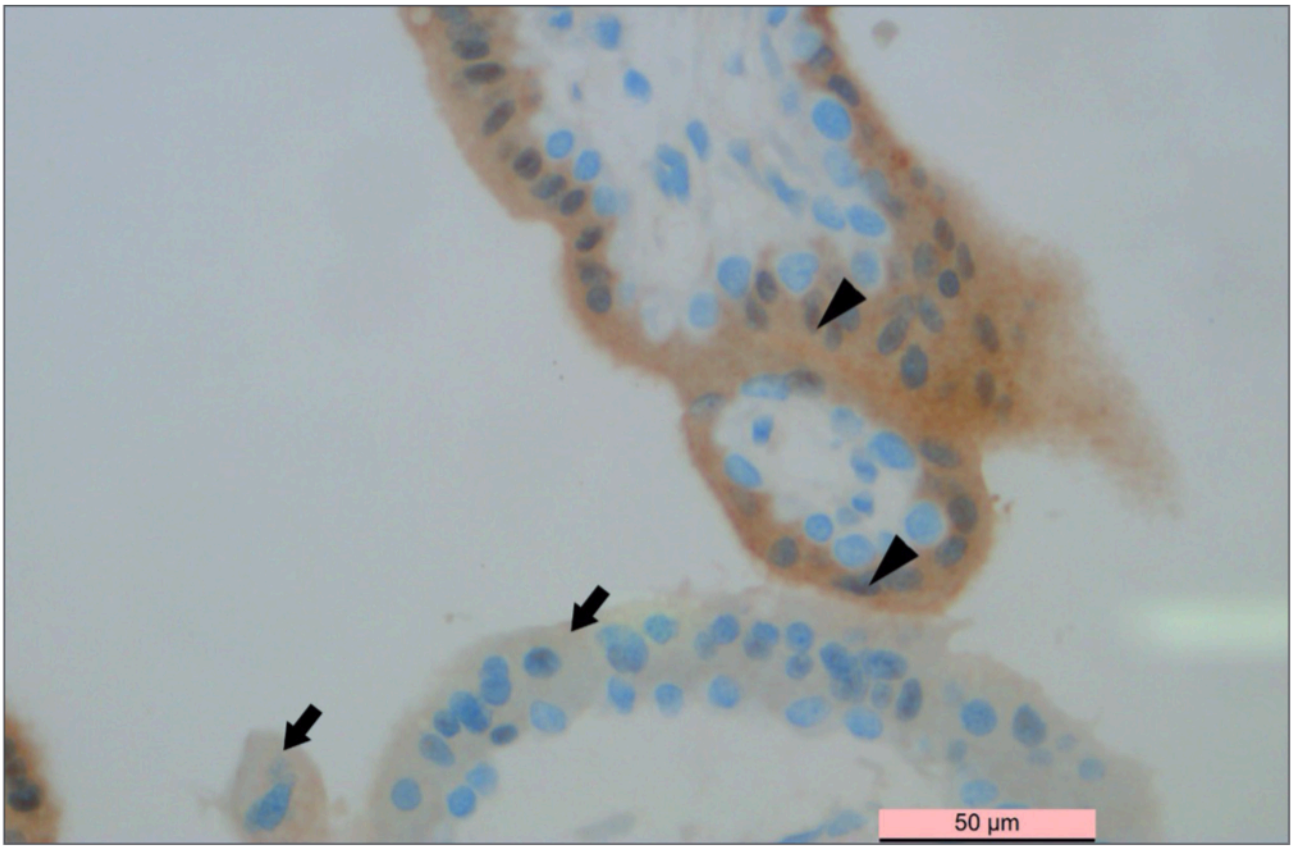

Figure 6. Evident cytoplasmic (arrows) and nuclear (arrowheads) immunolabeling for HOPX.

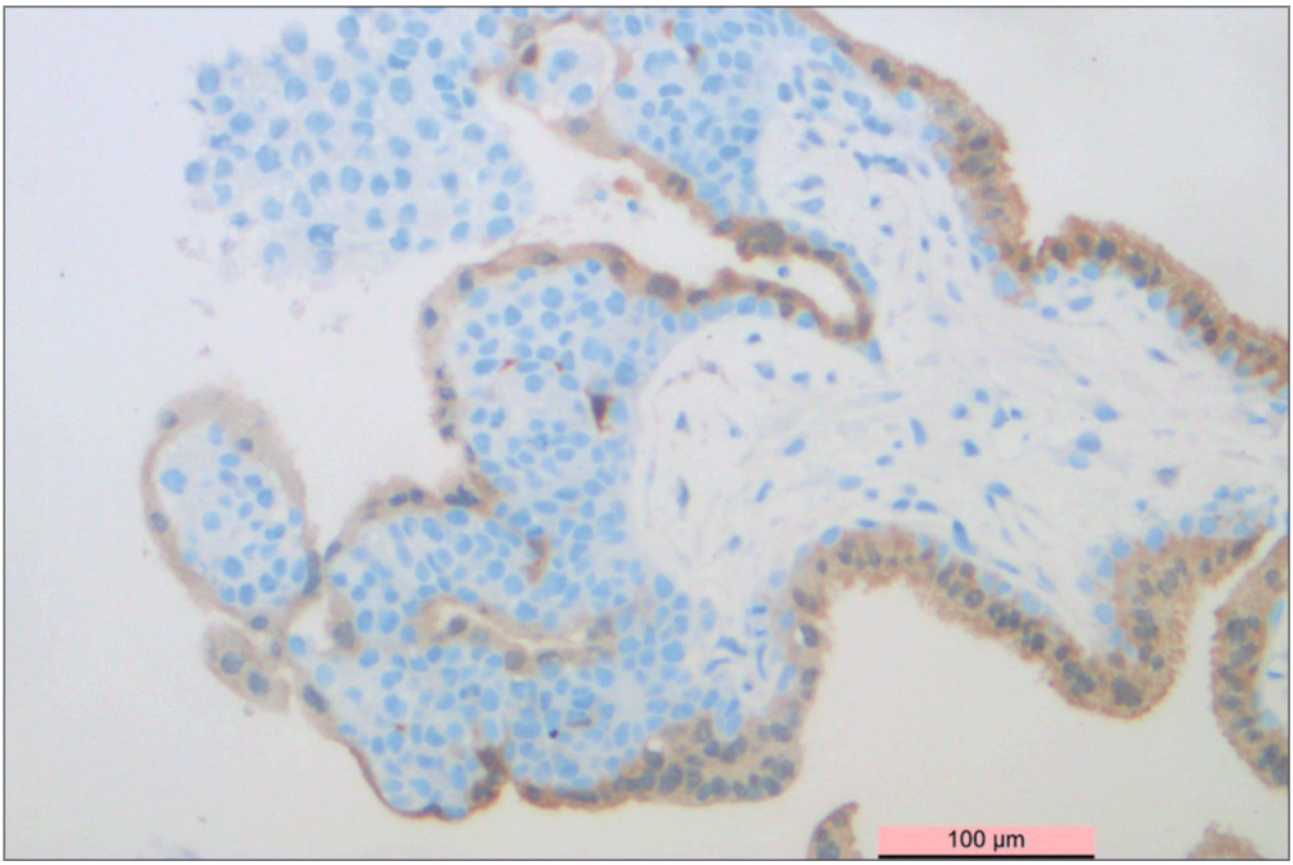

Figure 7. Absence of immunolabeling for HOPX in immature trophoblasts.

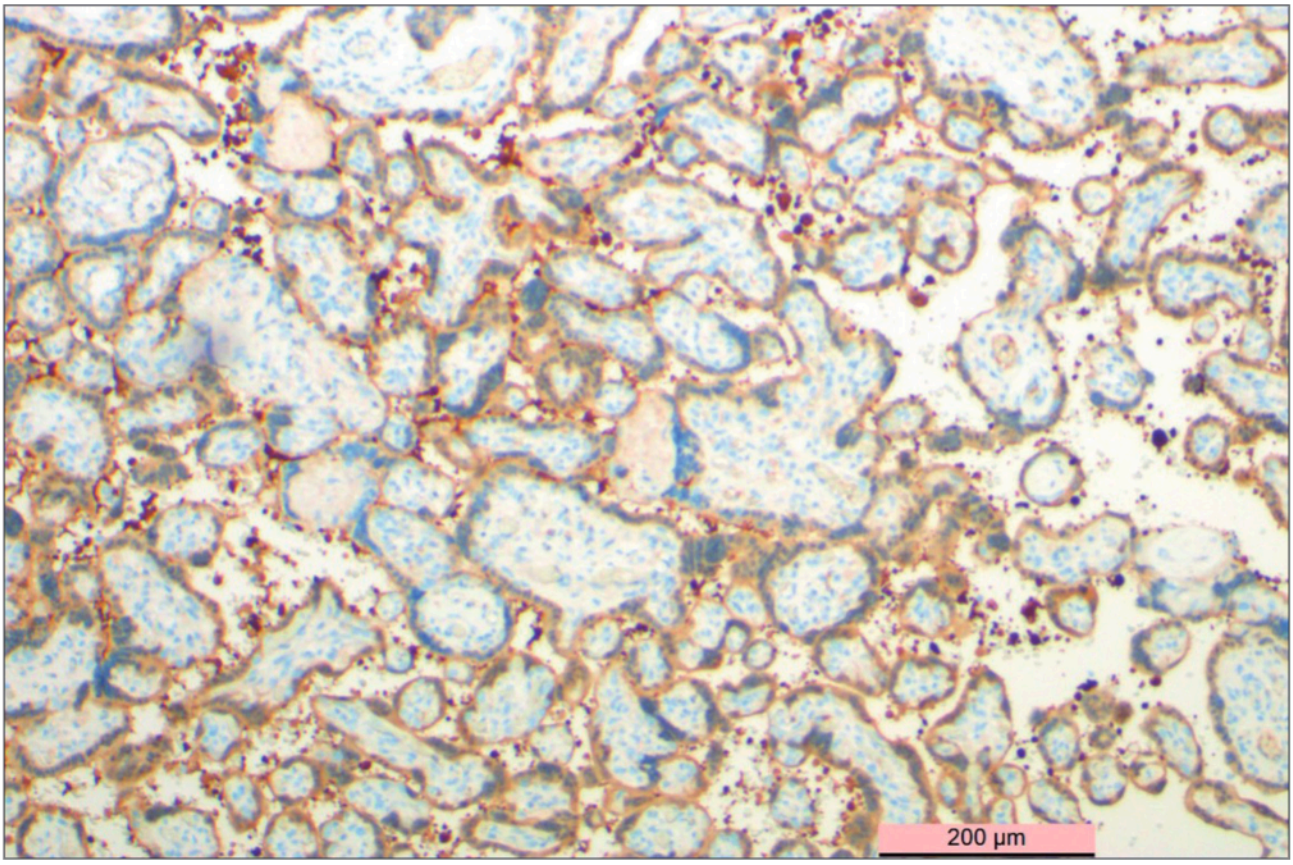

Figure 8. Panoramic view of a strong immunolabeling for HOPX inside the inter-villous spaces and reduced immunolabeling in some syncytiotrophoblast cells.

Discussion

Successful pregnancy needs a carefully coordinated relationship between the mother and the fetus, able to promote healthy pregnancy and optimal fetal development [12]. Pregnancy immune tolerance at the maternal-fetal interface represents a very important topic in human physiology and in immunology [13]. Multiple mechanisms have been proposed, during the years, to explain this tolerance. Decidual NK cells have been indicated as important players in the immune microenvironment at the maternal-fetal interface [14, 15]. The mechanisms underlying maternal acceptance of the fetus have not been fully clarified yet [10].

HOPX is expressed during embryonic development in several organs, including placenta [16]. HOPX plays a key role in placental development, controlling multiple transcription factors involved in trophoblast differentiation, inducing the differentiation of placenta stem/progenitor cells into a trophoblastic lineage [17].

In this study, we show that syncytiotrophoblasts, the cells covering the placental villi originating by fusion of underlying progenitor cells called cytotrophoblasts, are characterized by a strong immunoreactivity for HOPX, that has been reported to act as a negative regulator of T-lymphocytes, including CD4+ T cells, inhibiting their activation [9]. These data, taken together, suggest the hypothesis that HOPX might participate to maternal tolerance of the developing embryo, by inactivating maternal T-lymphocytes present in the maternal blood trafficking in the intervillous spaces of the placenta. According with this hypothesis, HOPX could participate to the complex processes underlying maternal tolerance, allowing the development of the placenta and its fundamental function in embryonal and fetal physiological development. Peripherally-induced immune tolerance mainly depends on peripheral regulatory T cells that require high expression of HOPX to inhibit the expression of IL-2 [18].

The interindividual variability in HOPX expression detected in this preliminary study reinforces the hypothesis that changes in HOPX expression could be related to interindividual differences in maternal immune status. Moreover, patchy expression could be related to underlying subclinical maternal pathologies or to pathological events occurring during pregnancy, which could favor maternal non-acceptance of the fetus, due to the absence or decreased expression of this protein on the surface of placental villi. The questions related to the interindividual variability in placenta cells remain open. The cases analyzed in this study were all characterized by the absence of clinical and histological signs of maternal reactivity against fetal cells. Further studies are needed, to analyze the expression of HOPX in placentas from women who underwent abortion due to maternal immune intolerance.

Among the other findings emerging from this study, the observation of HOPX expression inside the nuclei of syncytiotrophoblast cells deserves some consideration. Since the proteins encoded by homeobox genes are transcription factors [4], their presence in the nuclei reflects their main activity as modulators of the activity of multiple genes involved in proliferation and differentiation during gestation. It remains to be better explained why HOPX, in the majority of cases, appears mainly localized in the cytoplasm, with a minor expression at nuclear level.

Another intriguing finding regards the observation of HOPX in extracellular vesicles, putatively secreted by syncytiotrophoblats into the intervillous spaces. The ability of syncytiotrophoblasts of secreting HOPX in the inter-villi spaces through extracellular vesicles is, at the best of our knowledge, a new finding that deserves some consideration. Our findings parallel recent data on the ability of spinal cell neurons to export L1 cell adhesion molecule (L1CAM) through extracellular vesicles during embryogenesis, favoring axon sprouting and branching [19]. The secretion of HOPX outside the cells covering placental villi could reinforce the ability of placental cells to block the activity of maternal T-lymphocytes circulating in the intervillous spaces. It remains to be clarified why HOPX-reactive extracellular vesicles were detected only in a small percentage of cases.

Conclusion

In conclusion, this study clearly shows the expression of HOPX in the human placenta, mainly localized in the cytoplasm and nuclei of syncytiotrophoblast cells, at the border between fetal placental villi and maternal blood. The putative role of HOPX protein in down-regulating the activity of maternal T-lymphocytes, participating to maternal tolerance against the embryo and fetus, necessitates further studies, aimed to evaluate differences in HOPX expression

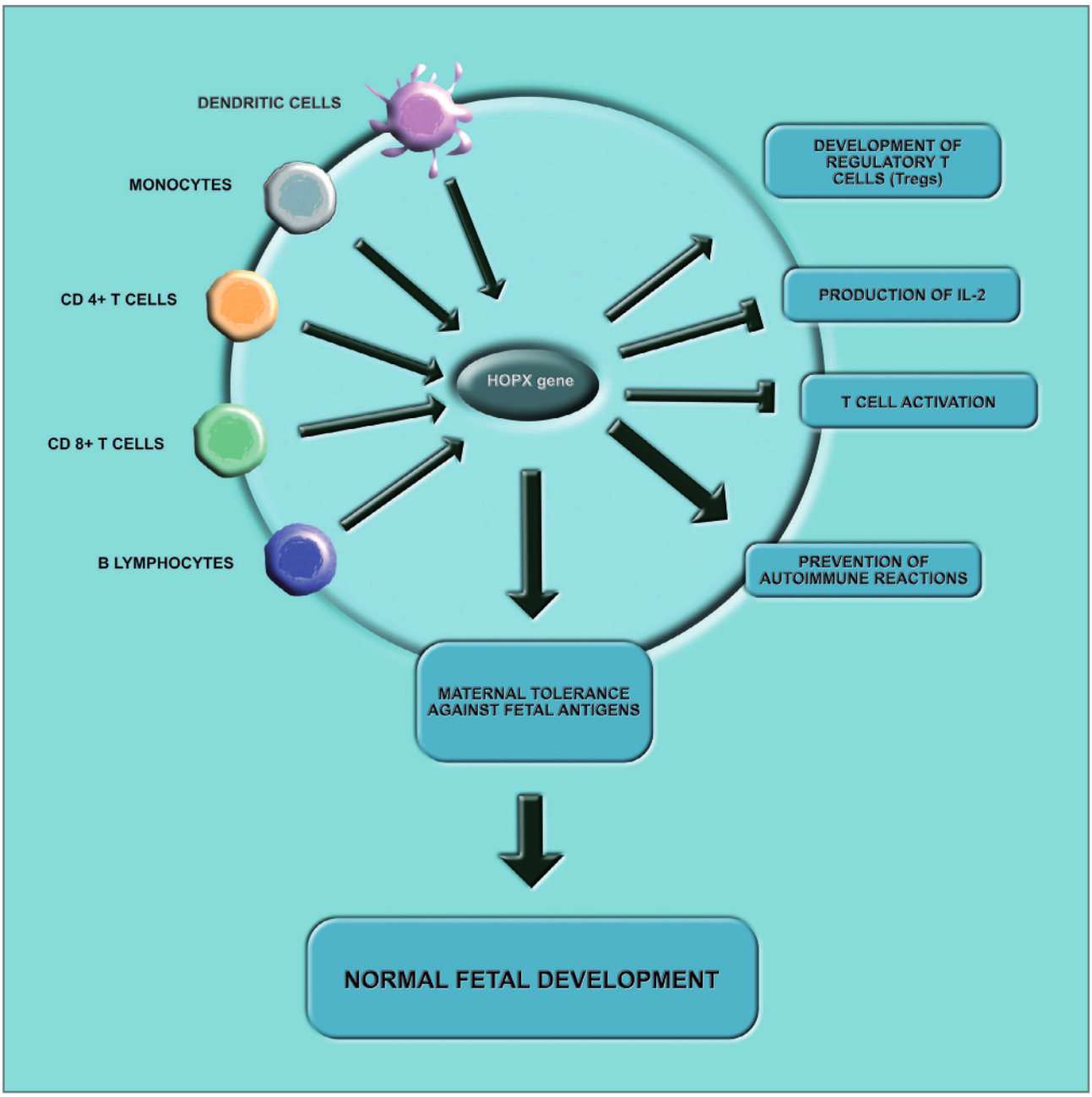

Figure 9. Role of homeodomain-only protein homeobox (HOPX) in fetal development.

in normal pregnancies compared with abortive pregnancy related to maternal non-acceptance of the fetus (**Fig. 9**). Further clinical-pathological studies are needed to integrate placental HOPX expression during gestation with placental immunological processes, in order to demonstrate the link between placental HOPX expression, immunological mechanisms, and the clinical implications of HOPX dysregulation, including preeclampsia and recurrent miscarriage.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Taylor HS. The role of HOX genes in the development and function of the female reproductive tract. Semin Reprod Med. 2000;18(1):81-90.
2. McGinnis W, Krumlauf R. Homeobox genes and axial patterning. Cell. 1992;68(2):283-302.
3. Holland PWH. Evolution of homeobox genes. Wiley Interdiscip Rev Dev Biol. 2013;2(1):31-45.
4. Abate-Shen C. Deregulated homeobox gene expression in cancer: Cause or consequence? Nat Rev Cancer. 2002;2:777-85.
5. Kook H, Yung WW, Simpson RJ, Kee HJ, Shin S, Lowry JA, Loughlin FE, Yin Z, Epstein JA, Mackay JP. Analysis of the structure and function of the transcriptional coregulator HOP. Biochemistry. 2006;45(35):10584-90.

6. Mühlfriedel S, Kirsch F, Gruss P, Stoykova A, Chowdhury K. A roof plate-dependent enhancer controls the expression of Homeodomain only protein in the developing cerebral cortex. Dev Biol. 2005;283(2):522-34.
7. Jones A, Kainz D, Khan F, Lee C, Carrithers MD. Human macrophage SCN5A activates an innate immune signaling pathway for antiviral host defense. J Biol Chem. 2014;289(51):35326-40.
8. Bourque J, Opejin A, Surnov A, Iberg CA, Gross C, Jain R, Epstein JA, Hawiger D. Landscape of Hopx expression in cells of the immune system. Heliyon. 2021;7(11):e08311.
9. Yang Q, Patrick M, Lu J, Chen J, Zhang Y, Hemani H, Lehrmann E, De S, Weng N. Homeodomain-only protein suppresses proliferation and contributes to differentiation- and age-related reduced CD8+ T cell expansion. Front Immunol. 2024;15:1360229.
10. Guleria I, Sayegh MH. Maternal Acceptance of the Fetus: True Human Tolerance. J Immunol. 2007;178(6):3345-51.
11. Yamashita K, Katoh H, Watanabe M. The homeobox only protein homeobox (HOPX) and colorectal cancer. Int J Mol Sci. 2013;14(12):23231-43.
12. Yockey LJ, Iwasaki A. Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. Immunity. 2018;49(3):397-412.
13. Li X, Zhou J, Fang M, Yu B. Pregnancy immune tolerance at the maternal-fetal interface. Int Rev Immunol. 2020;39(6):247-63.
14. Fu B, Wei H. Decidual natural killer cells and the immune microenvironment at the maternal-fetal interface. Sci China Life Sci. 2016;59:1224-31.
15. Liu Y, Gao S, Zhao Y, Wang H, Pan Q, Shao Q. Decidual Natural Killer Cells: A Good Nanny at the Maternal-Fetal Interface During Early Pregnancy. Front Immunol. 2021;12:663660.
16. Asanoma K, Kato H, Yamaguchi S, Shin CH, Liu ZP, Kato K, Inoue T, Miyanari Y, Yoshikawa K, Sonoda K, Fukushima K, Wake N. HOP/NECC1, a novel regulator of mouse trophoblast differentiation. J Biol Chem. 2007;282(33):24065-74.
17. Jaremek A, Jeyarajah MJ, Jaju Bhattad G, Renaud SJ. Omics Approaches to Study Formation and Function of Human Placental Syncytiotrophoblast. Front Cell Dev Biol. 2021;9:674162.
18. Jones A, Opejin A, Henderson JG, Gross C, Jain R, Epstein JA, Flavell RA, Hawiger D. Peripherally Induced Tolerance Depends on Peripheral Regulatory T Cells That Require Hopx To Inhibit Intrinsic IL-2 Expression. J Immunol. 2015;195(4):1489-97.
19. Cau F, Fanni D, Manchia M, Gerosa C, Piras M, Murru R, Paribello P, Congiu T, Coni P, Pichiri G, Piludu M, Van Eyken P, Gibo Y, La Nasa G, Orrù G, Scano A, Coghe F, Saba L, Castagnola M, Faa G. Expression of L1 Cell Adhesion Molecule (L1CAM) in extracellular vesicles in the human spinal cord during development. Eur Rev Med Pharmacol Sci. 2022;26(17):6273-82.