Advances in Clinical Medicine 临床医学进展, 2023, 13(4), 6389-6393
Published Online April 2023 in Hans. https://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2023.134898

胎儿微嵌合细胞及抗原通过外泌体途径调节HLA-C影响复发性流产

卢冲冲[1,2]，王　珊[1,2*]

[1]山东大学，山东　济南
[2]山东省立医院，山东　济南

收稿日期：2023年3月21日；录用日期：2023年4月17日；发布日期：2023年4月25日

摘　要

复发性流产作为妇产及生殖领域一疑难病症，主要表现为2次及以上的自然流产，其发病机制广泛而复杂。主要涉及染色体、母胎免疫等各方面原因。胚胎作为一种半同种异体移植物，其自身细胞及抗原可通过胎盘循环进入母体进而调节母体的免疫功能。外泌体作为一种直径在40~160 nm之间的囊泡，可作为运输载体包裹蛋白、非编码RNA等多种物质来实现细胞间的信号传导及物质传递。母体与胚胎免疫识别依靠HLA家族的不同亚型来实现，而HLA-C是唯一可在滋养细胞表面表达的MHC I类分子，其可作为杀伤细胞免疫球蛋白样受体的配体发挥免疫调节作用。综上所述，胎儿微嵌合细胞及抗原可通过外泌体途径调节HLA-C影响复发性流产的产生及进展。

关键词

复发性流产，胎儿微嵌合(FMC)，外泌体，HLA-C

Effect of Fetal Microchimeristic Cells and Antigens on Recurrent Spontaneous Abortion by Regulating HLA-C through Exocrine Pathway

Chongchong Lu[1,2], Shan Wang[1,2*]

[1]Shandong University, Jinan Shandong
[2]Shandong Provincial Hospital, Jinan Shandong

[*]通讯作者。

文章引用: 卢冲冲, 王珊. 胎儿微嵌合细胞及抗原通过外泌体途径调节HLA-C影响复发性流产[J]. 临床医学进展, 2023, 13(4): 6389-6393. DOI: 10.12677/acm.2023.134898

Received: Mar. 21st, 2023; accepted: Apr. 17th, 2023; published: Apr. 25th, 2023

Abstract

As a difficult disease in the field of gynecology, obstetrics and reproduction, recurrent spontaneous abortion is mainly manifested as two or more miscarriages, and its pathogenesis is extensive and complex. It mainly involves chromosome, maternal and fetal immunity and other reasons. Embryo, as a kind of semi-allogeneic graft, its own cells and antigens can enter the mother through the placenta circulation to regulate the mother's immune function. As a kind of vesicle with a diameter of 40~160 nm, exosomes can be used as a transport carrier to wrap protein, non-coding RNA and other substances to realize signal transmission and material transmission between cells. Mothers and embryos rely on different subtypes of HLA family to realize, and HLA-C is the only MHC molecule that can be expressed on the surface of trophoblasts, which can play an immunomodulatory role as a ligand of killer cell immunoglobulin-like receptor. To sum up, fetal microchimeric cells and antigens can regulate HLA-C through the exocrine pathway to affect the generation and progress of recurrent abortion.

Keywords

Recurrent Spontaneous Abortion (RSA), Fetal Microchimerism (FMC), Exosomes, HLA-C

1. 引言

复发性自然流产(recurrent spontaneous abortion, RSA)是指 2 次及 2 次以上的自然流产[1]，病因多而复杂，其中半数病因不清，称为不明原因复发性流产(unexplained recurrent spontaneous abortion, URSA)。URSA 为临床常见疑难病，人群发病率约为 1%，其中约 50%与免疫因素相关[2]。免疫学认为，胚胎含有父方和母方各一半的基因，在子宫蜕膜着床后其作为一种同种半异体移植物未受到母体免疫系统攻击是由母－胎免疫耐受经准控制的[3] [4]。至今妊娠期间诱导产生免疫耐受的详细机制尚未阐明，URSA 的诊断缺乏特异性检测指标，各种治疗方法如抗凝疗法、免疫球蛋白输注、淋巴细胞免疫治疗等均存在较大的争议[5] [6]。临床亟需开发新的有效诊治手段，改善患者妊娠结局。

妊娠期胎儿细胞可经胎盘屏障进入母体，形成嵌合状态。嵌合体是指遗传物质不同的两种及以上的细胞系存在于同一机体的现象。常见的导致机体嵌合状态的原因有妊娠、器官移植以及输血。其中妊娠及部分双胎中嵌合细胞所占比例少于每 10^4~10^5 细胞中含有 1 个不同遗传物质的细胞，被称为微嵌合。近年研究发现，母体胎儿细胞的微嵌合(fetal microchimerism, FMC)在调节母胎界面免疫平衡方面具有重要作用[7]。胎盘滋养细胞是妊娠期微嵌合细胞的主要来源，微嵌合滋养细胞调节母胎免疫的具体机制可能涉及基因表达调控、细胞间信号传递等多层面[5]，具有极大的研究价值。

2. 胎儿微嵌合调节母体免疫且与复发性流产等多种不良妊娠相关

随着胚胎发育逐渐形成胎盘，其与母体重铸的子宫螺旋动脉形成的母－胎屏障既可以保证物质交换，同时又能够避免母－胎发生免疫排斥，是形成微嵌合体现象的基础。其具体的形成机制尚不清楚，免疫

耐受及致敏可能在 FMC 形成及其在相关疾病的变化方面发挥重要作用[8]。1996 年 Bianchi 等[9]首次发现了在初次分娩男性胎儿的孕妇体内存在男性的免疫细胞，并且确定了其来自分娩的胎儿，于此同时他们检测母体器官 FMC 含量发现除血液外其可广泛分布于肝、脾、甲状腺、淋巴等部位。在早期妊娠，胎儿细胞或其他物质即可进入母体组织。胎儿细胞最早在妊娠 4~6 周即可检出，妊娠中期时母体每毫升血液中约含 1~6 个胎儿细胞，几乎所有女性在晚期妊娠后都可以检出。分娩以后胎儿微嵌合体(FMC)数量逐渐下降并趋于稳定，但其在母体内可长期存在[10]。研究认为这些 FMC 的存在可调节母－胎免疫，作为同种半异体抗原的载体，FMC 可调节机体体液及细胞免疫，增加母体对父方抗原耐受性，对胚胎的正常存活及发育起到重要调节作用[11]。

绝大多数妊娠妇女体内都存在 FMC，其异常可能与子痫前期等妊娠期并发症以及胚胎停育、自然流产等不良孕产史有关[7] [12]；胎儿抗原诱导调节 T 细胞转化及 NK 细胞杀伤活性降低等，可能导致了多发性硬化症(multiple sclerosis, MS)和类风湿性关节炎(rheumatoid arthritis, RA)等自身免疫性疾病在孕期得到改善，而其他疾病如系统性红斑狼疮(systemic lupus erythematosus, SLE)则恶化[13]。Sato 等[14]发现，与自然流产者相比，人工流产患者由于清宫等宫腔内的有创操作，其体内检测出更多的 FMC，提示了不良孕产史与 FMC 的相关性。有学者认为，经产妇发生自然流产的几率远低于初产妇，可能的机制之一为胎儿抗原在前次妊娠诱导母体记忆性 T 细胞的耐受[15]。阐明微嵌合细胞或抗原在母胎免疫耐受诱导过程中的作用机制，有望为预防和治疗胚胎停育及保胎等提供新的临床思路。

3. 微嵌合细胞或抗原可通过外泌体途径调节生殖免疫

2015 年《cell》[16]发文指出母体通过来自胎儿时期由祖母循环获得的微嵌合细胞诱导调节 T 细胞识别及接受这些非遗传性的微嵌合抗原阻止了免疫系统排斥胎儿，研究组胚胎丢失率显著低于对照组，母体内的微嵌合抗原有利于跨代生殖健康。该文证实了妊娠中微嵌合抗原重要的免疫调节作用，为阐明母胎免疫耐受机制开辟了新的思路。

新近研究[17]表明，微嵌合量级足以引起宿主树突细胞(Dendritic cells, DC)的膜同种异体抗原识别(mAAQ; “cross-dressing”)。来自 mAAQ+，而不是来自非 mAAQ 的小鼠的富含胞外囊泡(EV)的血清在体外再现 DC 反串现象。在体内，mAAQ 与 DC 的免疫检查点分子 PD-L1 和共刺激分子 CD86 的表达增加以及同种异体肽+自身 MHC 复合物的表达减少相关。William [17]等通过追踪外源特异性转基因 CD4+ T 细胞的分化揭示了微嵌合小鼠的“分裂耐受性”状态：能够识别完整获得的 MHC 同种异体抗原的 T 细胞增殖，而识别自身 MHC 的 T 细胞没有增殖。这表明同一树突状细胞通过外泌体途径获得微嵌合抗原，这些同种异体抗原可诱导产生不同的 T 细胞反应，从而产生完全相反的免疫应答。因此，我们有理由推测微嵌合抗原(或细胞)可通过外泌体途径调节免疫细胞功能。

4. 胎盘外泌体可调节 NK 细胞功能极化，微嵌合细胞或抗原表达可能在其中发挥作用

外泌体是胞外囊泡的一种，直径大约在 40~160 nm 之间(平均 100 nm)，含有丰富的生物活性物质：蛋白质、脂质、mRNA、微小 RNA (microRNA, miRNA)和长链非编码 RNA (longnoncoding RNA, lncRNA)等。外泌体可与多种细胞作用，把其内容物运送及释放至靶细胞中，发挥相应调控作用。

外泌体具有重要的免疫调节功能[18]，在母胎免疫中发挥重要的作用，其浓度和生物活性与复发性流产、子痫前期、妊娠期糖尿病、早产等多种病理妊娠的发病有关，涉及母体免疫调节、螺旋动脉重塑、炎症反应等病理生理过程[19] [20]。妊娠 6 周后即可在母体血液中检测到胎盘外泌体[21]，并且随着孕周的增加孕妇体内的外泌体数量不断增加，在妊娠晚期达到高峰[22]。妊娠期微嵌合细胞以滋养细胞为主，滋养细胞是妊娠早期胎盘外泌体的主要来源[23]，而且，滋养细胞来源外泌体可表达并存储针对 NKG2D

的配体 MHC I 类抗原 HLA-A、B 分子，介导 NK 细胞和细胞毒性 T 细胞凋亡，是细胞表面缺乏 HLA-A、B 表达的滋养细胞母胎免疫逃逸的机制之一[24] [25]。而 HLA-C 是唯一可在滋养细胞表面表达的经典的 MHC I 类分子[26]，且滋养细胞可检测到 HLA-C 父系等位基因序列的表达[27]。

HLA-C 研究的重要进展是其可作为杀伤细胞免疫球蛋白样受体(killer cell immunoglobulin-like receptors, KIRs)的配体发挥免疫调节作用[28]。根据 KIR 特异性识别位点的不同，HLA-C 可分为 2 组：HLA-Cw01、03、07、08 分子 α 重链上第 80 位的氨基酸为天冬氨酸，称为第 I 组(HLA-C1)；HLA-Cw02、04、05、06 分子 α 重链上第 80 位为赖氨酸，称为第 II 组(HLA-C2) [29]。KIR 具有高度的物种特异性，在母胎界面上发挥重要作用[30]，KIR2DL1 为 KIR 超家族一员，胞内段含有酪氨酸抑制基序(Immunoreceptor tyrosine-based activation motif, ITIM)，与靶细胞 HLA-C2 识别后传导抑制性信号。研究发现母体 NK 细胞，特别是大部分蜕膜 NK 细胞表达 HLA-C 特异性 KIR 如 KIR2DL1/S1 [31]，这提示了蜕膜 NK 细胞 KIR2DL1 受体与滋养细胞 HLA-C 的识别可能参与诱导胚胎免疫耐受。

综上所述胚胎是一种同种半异体移植物，其可通胎盘等结构将少量自身细胞及其他物质融入母体形成母胎微嵌合状态，并且其可调节母体的免疫应答从而避免自身受到攻击。妊娠期母体 NK 细胞 KIR 与滋养细胞 HLA 抗原的识别启动了特殊的免疫应答，外泌体作为循环细胞及局部微环境细胞间对话的重要媒介，介导了妊娠期 NK 细胞和滋养细胞间信号传导发挥了调节作用。

参考文献

[1] The Practice Committee of the American Society for Reproductive Medicine (2012) Evaluation and Treatment of Recurrent Pregnancy Loss: A Committee Opinion. *Fertility and Sterility*, **98**, 1103-1111. https://doi.org/10.1016/j.fertnstert.2012.06.048

[2] Tur-Torres, M.H., Garrido-Gimenez, C. and Alijotas-Reig, J. (2017) Genetics of Recurrent Miscarriage and Fetal Loss. *Best Practice & Research Clinical Obstetrics & Gynaecology*, **42**, 11-25. https://doi.org/10.1016/j.bpobgyn.2017.03.007

[3] Yang, F., Zheng, Q. and Jin, L. (2019) Dynamic Function and Composition Changes of Immune Cells during Normal and Pathological Pregnancy at the Maternal-Fetal Interface. *Frontiers in Immunology*, **10**, Article 2317. https://doi.org/10.3389/fimmu.2019.02317

[4] Erlebacher, A. (2013) Immunology of the Maternal-Fetal Interface. *Annual Review of Immunology*, **31**, 387-411. https://doi.org/10.1146/annurev-immunol-032712-100003

[5] Zhao, L., Bi, S.Q., Fu, J.H., Qi, L.J., Li, L. and Fu, Y.H. (2021) Retrospective Analysis of Fondaparinux and Low-Molecular-Weight Heparin in the Treatment of Women with Recurrent Spontaneous Abortion. *Frontiers in Endocrinology*, **12**, Article 717630. https://doi.org/10.3389/fendo.2021.717630

[6] Alijotas-Reig, J., Esteve-Valverde, E., Ferrer-Oliveras, R., Llurba, E. and Gris, J.M. (2017) Tumor Necrosis Factor-Alpha and Pregnancy: Focus on Biologics. An Updated and Comprehensive Review. *Clinical Reviews in Allergy & Immunology*, **53**, 40-53. https://doi.org/10.1007/s12016-016-8596-x

[7] Hahn, S., Hasler, P., Vokalova, L., van Breda, S.V., Than, N.G., Hoesli, I.M., Lapaire, O. and Rossi, S.W. (2019) Feto-Maternal Microchimerism: The Pre-Eclampsia Conundrum. *Frontiers in Immunology*, **10**, Article 659. https://doi.org/10.3389/fimmu.2019.00659

[8] Partha, D. and Burlingham, W.J. (2011) Microchimerism: Tolerance vs. Sensitization. *Current Opinion in Organ Transplantation*, **16**, 359-365. https://doi.org/10.1097/MOT.0b013e3283484b57

[9] Bianchi, D.W., Zickwolf, G.K., Weil, G.J., Sylvester, S. and DeMaria, M.A. (1996) Male Fetal Progenitor Cells Persist in Maternal Blood for as Long as 27 Years Postpartum. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 705-708. https://doi.org/10.1073/pnas.93.2.705

[10] Cómitre-Mariano, B., Martínez-García, M., García-Gálvez, B., Paternina-Die, M., Desco, M., Carmona, S. and Gómez-Gaviro, M.V. (2022) Feto-Maternal Microchimerism: Memories from Pregnancy. *iScience*, **25**, Article ID: 103664. https://doi.org/10.1016/j.isci.2021.103664

[11] Kinder, J.M., Stelzer, I.A., Arck, P.C. and Way, S.S. (2017) Immunological Implications of Pregnancy-Induced Microchimerism. *Nature Reviews Immunology*, **17**, 483-494. https://doi.org/10.1038/nri.2017.38

[12] Deshmukh, H. and Way, S.S. (2019) Immunological Basis for Recurrent Fetal Loss and Pregnancy Complications. *Annual Review of Pathology*, **14**, 185-210. https://doi.org/10.1146/annurev-pathmechdis-012418-012743

[13] Patas, K., Engler, J.B., Friese, M.A. and Gold, S.M. (2013) Pregnancy and Multiple Sclerosis: Feto-Maternal Immune Cross Talk and Its Implications for Disease Activity. *Journal of Reproductive Immunology*, **97**, 140-146. https://doi.org/10.1016/j.jri.2012.10.005

[14] Sato, T., Fujimori, K., Sato, A. and Ohto, H. (2008) Microchimerism after Induced or Spontaneous Abortion. *Obstetrics & Gynecology*, **112**, 593-597. https://doi.org/10.1097/AOG.0b013e31818345da

[15] Rowe, J.H., Ertelt, J.M., Xin, L.J. and Way, S.S. (2012) Pregnancy Imprints Regulatory Memory That Sustains Anergy to Fetal Antigen. *Nature*, **490**, 102-106. https://doi.org/10.1038/nature11462

[16] Kinder, J.M., Jiang, T.T., Ertelt, J.M., Xin, L.J., Strong, B.S., Shaaban, A.F. and Way, S.S. (2015) Cross-Generational Reproductive Fitness Enforced by Microchimeric Maternal Cells. *Cell*, **162**, 505-515. https://doi.org/10.1016/j.cell.2015.07.006

[17] Bracamonte-Baran, W., Florentin, J., Zhou, Y., Jankowska-Gan, E., Haynes, W. J., Zhong, W., Brennan, T.V., Dutta, P., Claas, F.H.J., van Rood, J.J. and Burlingham, W.J. (2017) Modification of Host Dendritic Cells by Microchimerism-Derived Extracellular Vesicles Generates Split Tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 1099-1104. https://doi.org/10.1073/pnas.1618364114

[18] Chaput, N. and Théry, C. (2011) Exosomes: Immune Properties and Potential Clinical Implementations. *Seminars in Immunopathology*, **33**, 419-440. https://doi.org/10.1007/s00281-010-0233-9

[19] Nair, S. and Salomon, C. (2018) Extracellular Vesicles and Their Immunomodulatory Functions in Pregnancy. *Seminars in Immunopathology*, **40**, 425-437. https://doi.org/10.1007/s00281-018-0680-2

[20] Salomon, C., Scholz-Romero, K., Sarker, S., Sweeney, E., Kobayashi, M., Correa, P., Longo, S., Duncombe, G., Mitchell, M.D., Rice, G.E. and Illanes, S.E. (2016) Gestational Diabetes Mellitus Is Associated with Changes in the Concentration and Bioactivity of Placenta-Derived Exosomes in Maternal Circulation Across Gestation. *Diabetes*, **65**, 598-609. https://doi.org/10.2337/db15-0966

[21] Bai, K.F., Xintong Li, X.T., Zhong, J.M., Ng, E.H.Y., Yeung, W.S.B., Lee, C.-L. and Chiu, P.C.N. (2021) Placenta-Derived Exosomes as a Modulator in Maternal Immune Tolerance during Pregnancy. *Frontiers in Immunology*, **12**, Article 671093. https://doi.org/10.3389/fimmu.2021.671093

[22] Sarker, S., Scholz-Romero, K., Perez, A., Illanes, S.E., Mitchell, M.D., Rice, G.E. and Salomon, C. (2014) Placenta-Derived Exosomes Continuously Increase in Maternal Circulation over the First Trimester of Pregnancy. *Journal of Translational Medicine*, **12**, Article No. 204. https://doi.org/10.1186/1479-5876-12-204

[23] Tong, M. and Chamley, L.W. (2015) Placental Extracellular Vesicles and Feto-Maternal Communication. *Cold Spring Harbor Perspectives in Medicine*, **5**, a023028. https://doi.org/10.1101/cshperspect.a023028

[24] Mincheva-Nilsson, L. (2021) Immunosuppressive Protein Signatures Carried by Syncytiotrophoblast-Derived Exosomes and Their Role in Human Pregnancy. *Frontiers in Immunology*, **12**, Article 717884. https://doi.org/10.3389/fimmu.2021.717884

[25] Tersigni, C., Meli, F., Neri, C., Iacoangeli, A., Franco, R., Lanzone, A., Scambia, G. and Di Simone, N. (2020) Role of Human Leukocyte Antigens at the Feto-Maternal Interface in Normal and Pathological Pregnancy: An Update. International Journal of Molecular Science, **21**, Article No. 4756. https://doi.org/10.3390/ijms21134756

[26] Meuleman, T., Haasnoot, G.W., van Lith, J.M.M., Verduijn, W., Bloemenkamp, K.W.M. and Claas, F.H.J. (2018) Paternal HLA-C Is a Risk Factor in Unexplained Recurrent Miscarriage. American Journal of Reproductive Immunology, **79**, e12797. https://doi.org/10.1111/aji.12797

[27] Papúchová, H., Meissner, T.B., Li, Q., Strominger, J.L. and Tilburgs, T. (2019) The Dual Role of HLA-C in Tolerance and Immunity at the Maternal-Fetal Interface. Frontiers in Immunology, **10**, Article 2730. https://doi.org/10.3389/fimmu.2019.02730

[28] Wang, S., Zhao, Y.-R., Jiao, Y.-L., Wang, L.-C., Li, J.-F., Cui, B., Xu, C.-Y., Shi, Y.-H. and Chen, Z.-J. (2007) Increased Activating Killer Immunoglobulin-Like Receptor Genes and Decreased Specific HLA-C Alleles in Couples with Recurrent Spontaneous Abortion. Biochemical and Biophysical Research Communications, **360**, 696-701. https://doi.org/10.1016/j.bbrc.2007.06.125

[29] Sim, M.J.W., Malaker, S.A., Khan, A., Stowell, J.M., Shabanowitz, J., Peterson, M.E., Rajagopalan, S., Hunt, D.F., Altmann, D.M., Long, E.O. and Boyton, R.J. (2017) Canonical and Cross-Reactive Binding of NK Cell Inhibitory Receptors to HLA-C Allotypes Is Dictated by Peptides Bound to HLA-C. Frontiers in Immunology, **8**, Article 193. https://doi.org/10.3389/fimmu.2017.00193

[30] Djaoud, Z. and Parham, P. (2020) HLAs, TCRs, and KIRs, a Triumvirate of Human Cell-Mediated Immunity. Annual Review of Biochemistry, **89**, 717-739. https://doi.org/10.1146/annurev-biochem-011520-102754

[31] Díaz-Hernández, I., Alecsandru, D., García-Velasco, J.A. and Domínguez, F. (2021) Uterine Natural Killer Cells: From Foe to Friend in Reproduction. Human Reproduction Update, **27**, 720-746. https://doi.org/10.1093/humupd/dmaa062