

炎症反应在多壁碳纳米管致胸膜间皮细胞恶性转化中的作用

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摘要:

[目的] 探讨巨噬细胞产生的炎症反应在多壁碳纳米管(multi-walled carbon nanotubes, MWCNTs)致胸膜间皮细胞恶性转化中的作用及可能的分子机制。

[方法] 分别在有(+)或无(-)MWCNTs染毒情况下,利用人胸膜间皮细胞(Met5A)和巨噬细胞共培养以及Met5A单独培养两种模式培养细胞3个月后,检测各组胸膜间皮细胞的增殖、迁移、克隆形成能力,及胸膜间皮细胞核因子- κ B(NF- κ B)、白细胞介素-6(IL-6)、信号转导和转录激活因子3(STAT3)基因表达水平的变化。

[结果] 与单独培养(+)组和单独培养(-)组比较,共培养(+)组间皮细胞的光密度值、穿膜的细胞数目、形成的细胞克隆数目均明显增加,差异具有统计学意义($P<0.05$)。单独培养(+)组与单独培养(-)组比较,间皮细胞的光密度值、穿膜的细胞数目、形成的细胞克隆数目增加,差异具有统计学意义($P<0.05$)。与单独培养(+)组及单独培养(-)组比较,共培养(+)组间皮细胞的NF- κ B、IL-6、STAT3基因表达水平明显升高,差异具有统计学意义($P<0.05$)。

[结论] MWCNTs可促进胸膜间皮细胞的恶性转化,且巨噬细胞产生的炎症反应可增强MWCNTs致胸膜间皮细胞的恶性转化能力。

关键词: 多壁碳纳米管; 巨噬细胞; 炎症反应; 胸膜间皮细胞; 恶性转化

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Role of inflammation in malignant transformation of pleural mesothelial cells induced by multi-walled carbon nanotubes HUANG Xiao-pei^a, XUE Wei^a, DONG Yi-fan^a, TIAN Yi-jun^a, ZHANG Xiao-fang^a, CHANG Wen-jun^b, ZHANG Tian-bao^a, ZHU Jiang-bo^a (a.Department of Health Toxicology b.Department of Environmental Hygiene, The Second Military Medical University, Shanghai 200433, China). Address correspondence to ZHU Jiang-bo, E-mail: jiangbozhu1@163.com • The authors declare they have no actual or potential competing financial interests.

Abstract:

[Objective] To investigate the role of inflammation caused by macrophages and the possible mechanisms in the malignant transformation of pleural mesothelial cells induced by multi-walled carbon nanotubes (MWCNTs).

[Methods] The proliferation, migration and colony formation ability of pleural mesothelial cells and the gene expression levels of nuclear factor kappa B (NF- κ B), interleukin 6 (IL-6), and signal transducer and activator of transcription 3 (STAT3) in pleural mesothelial cells were detected after three months of co-culture of mesothelial cells Met5A and macrophages and the separate culture of Met5A cells in the presence (+) or absence (-) of MWCNTs.

[Results] The proliferation, migration, and colony formation ability of mesothelial cells in the co-culture (+) group were significantly enhanced compared with those in the separate culture (+) group and the separate culture (-) group ($P<0.05$). The three indicators in the co-culture (+) group were also enhanced compared with those of the separate culture (-) group ($P<0.05$). The gene expression levels of NF- κ B, IL-6, and STAT3 in the co-culture (+) group were significantly increased compared with those in the separate culture (+) group and the separate culture (-) group ($P<0.05$).

[Conclusion] MWCNTs can promote malignant transformation of pleural mesothelial cells, furthermore this effect can be enhanced by the inflammation caused by macrophages.

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Keywords: multi-walled carbon nanotube; macrophage; inflammation; pleural mesothelial cell; malignant transformation

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胸膜间皮瘤是一种主要起源于胸膜壁层的恶性肿瘤,其起病隐匿,确诊时多已是晚期;在现行的治疗方式下,患者生存时间短,中位生存期为6—8月左右^[1]。石棉是目前唯一明确的致胸膜间皮瘤的环境因子^[2]。多壁碳纳米管(multi-walled carbon nanotubes, MWCNTs)是一种碳簇家族新成员,其结构可看成由碳原子形成的多层同心石墨烯片卷成的无缝、中空的管状物质,因具有良好的导电、导热、抗高温、耐腐蚀等性能而广泛应用于各个领域^[3]。MWCNTs在纤维特征上与石棉相似^[4-5],均符合WHO规定的“病理性纤维”的前提条件:高生物学持久性、纤维长度大于5 μm、纤维直径小于3 μm、纤维纵横比大于3:1。另外,MWCNTs以不同浓度存在于生产、加工场所的空气中,与石棉相同,吸入接触是其最主要的暴露途径^[6-7]。许多研究^[8-10]表明MWCNTs可诱发小鼠胸膜间皮瘤,但其致小鼠胸膜间皮瘤的分子机制尚不清楚。因此,对MWCNTs致胸膜间皮瘤作用及其毒性作用分子机制进行研究,既可为纳米材料从业人员的职业安全防护提供科学依据,又可为间皮瘤的临床治疗提供新的思路和借鉴。

目前认为纳米材料可能的遗传毒作用机制有两种^[11-12]:其一,直接作用于DNA或有丝分裂器而导致的直接遗传毒效应;其二,诱导细胞发生炎症反应,产生间接遗传毒效应。诱导炎症反应是目前较公认的生物学效应^[13]。有研究表明,MWCNTs单独作用于胸膜间皮细胞可引起胸膜间皮细胞的恶性转化。另外,MURPHY等^[14]的研究结果表明,长度较长的MWCNTs不能被巨噬细胞完全吞噬而出现吞噬受挫,产生炎症反应,释放大量的炎症因子,这些炎症因子可促进胸膜间皮细胞产生更强烈的促炎细胞因子的释放。炎症反应在肿瘤的发生、发展中具有重要作用^[15-16]。本研究拟探讨巨噬细胞产生的炎症反应对MWCNTs致胸膜间皮细胞恶性转化的影响。

1 材料与方法

1.1 材料

人胸膜间皮细胞Met5A及人单核细胞THP-1(ATCC,美国);胎牛血清、1640培养基及双抗(Gibco,

美国);MWCNTs(Cheaptubes,美国),直径30~50 nm,长度10~20 nm,纯度大于95%,灰分小于1.5%,比表面积60 m²/g;细胞培养皿、Transwell共培养小室(Corning,美国);细胞冻存液、胰蛋白酶(Thermo Fisher,美国);CCK8细胞增殖检测试剂盒(Sigma,美国);逆转录试剂盒(Invitrogen,美国);实时荧光定量PCR试剂盒(Takara,日本)。

1.2 方法

1.2.1 细胞毒性实验 Met5A细胞及THP-1细胞用含体积分数10%胎牛血清、体积分数1%双抗的Gibco 1640培养基,置于37℃、CO₂体积分数为5%、湿度为95%的培养箱中常规培养。待培养皿内细胞生长至80%左右传代,取对数期生长的细胞进行实验。实验设4组,分别为:Met5A单独培养组[单独培养(-)组],Met5A单独培养+MWCNTs组[单独培养(+)组],巨噬细胞+Met5A组[共培养(-)组],共培养+MWCNTs组[共培养(+)组]。共培养组是将含1×10⁶个THP-1细胞的2 mL培养基接种于共培养小室下室,加入0.4 μg佛波酯(phorbol-12-myristate-13-acetate, PMA),48 h后待其贴壁并分化为巨噬细胞后,更换为新鲜培养基,并在共培养小室上室加入含1.5×10⁵个Met5A细胞的1.5 mL培养基。待上室细胞生长至80%左右传代,继续共培养实验,共培养周期为3个月。单独培养组,在共培养小室上室加入含1.5×10⁵个Met5A细胞的培养基,下室加入新鲜培养基,待细胞生长至80%左右传代,培养3个月。MWCNTs溶于PBS,并加入肺表面活性剂固尔苏(可模拟体内天然的肺表面活性物质,对机体无毒),于超声中振荡,使其尽可能呈分散状态,以模拟其经呼吸道进入人体后在体内的存在状态。本研究使用浓度为0.1 μg/mL的MWCNTs作用于人胸膜间皮细胞,在此暴露水平下MWCNTs作用于间皮细胞3个月后可促进其恶性转化^[17]。

1.2.2 细胞增殖实验 将上述培养后的4组细胞用含体积分数10%胎牛血清的培养基配成单个细胞悬液,再将不同组的细胞悬液接种到96孔板中(4×10³个/孔),每组3个复孔,每孔体积100 μL,置于37℃、体积分数5%CO₂的培养箱中孵育。72 h后,每孔加CCK8溶液10 μL,孵育1.5 h。酶联免疫监测仪上测定450 nm

3 讨论

随着纳米材料的广泛应用, MWCNTs的致胸膜间皮瘤作用及其机制成为近年来研究的热点。LOHCHAROENKAL等^[17]的研究表明MWCNTs长期单独作用于胸膜间皮细胞可促进胸膜间皮细胞的恶性转化。本研究结果也表明, MWCNTs可使胸膜间皮细胞的增殖、迁移等恶性转化的能力增强, 与上述研究结果一致。另外, 炎症反应在MWCNTs致胸膜间皮细胞恶性转化中也起着重要作用。DONALDSON等^[18]综述前人的研究结果并提出假说, 长度较长的MWCNTs不能完全被巨噬细胞吞噬, 可产生炎症反应, 其释放的大量炎症因子可促进胸膜间皮瘤的形成。本结果表明, Met5A共培养(+)组细胞的增殖、迁移、克隆形成能力比Met5A单独培养(+)组明显增强, 共培养组既有MWCNTs对胸膜间皮细胞的直接作用, 也有巨噬细胞对MWCNTs吞噬受挫而产生的炎症反应的间接作用, 实验结果同样证明巨噬细胞产生的炎症反应在MWCNTs致胸膜间皮细胞的恶性转化中起着重要作用。

MWCNTs致胸膜间皮瘤的作用机制尚未完全阐明。已有的研究表明, MWCNTs可通过激活基质金属蛋白酶(MMP)-2等促进胸膜间皮细胞的恶性转化^[17]。MMP-2可以降解基底膜, 促进细胞外基质的重塑, 上调血管内皮生长因子的表达, 从而增强细胞的增殖、迁移、侵袭等能力^[17]。炎症反应产生的细胞因子可促进调节MMP-2的上游某些信号分子的激活。例如, 巨噬细胞不能完全吞噬MWCNTs, 产生的吞噬受挫伴随着氧化剂、细胞因子产生及溶酶体释放, 可促进大量的活性氧的产生^[19-20]。活性氧可以激活NF- κ B、蛋白激酶1等调控MMP-2的上游信号分子, 促进胸膜间皮细胞的恶性转化。MWCNTs刺激巨噬细胞产生的炎症反应还可促进胸膜间皮细胞释放大量的炎症因子, 以IL-6、TNF- α 等的释放量最多, 而IL-6又可激活核转录因子STAT3。许多研究表明, 转录因子STAT3可结合于MMP-2, 使其表达上调; 阻断STAT3的激活可抑制MMP-2的表达, 抑制肿瘤的生长、侵袭和转移等过程^[21]。MWCNTs致胸膜间皮瘤作用机制的更全面的分子网络有待进一步研究。

另外, 本研究结果显示, 在MWCNTs存在的条件下, 胸膜间皮细胞和巨噬细胞共培养3个月后, 胸膜间皮细胞的NF- κ B、IL-6、STAT3基因水平均明显增加。在乳腺癌和结肠癌中也存在着NF- κ B/IL-6/STAT3分子

通路, 被认为是自炎症到肿瘤转化的关键桥梁^[15-16]。NF- κ B作为关键因子, 可调控与细胞恶性转化(免疫逃避、侵袭、血管形成等)相关基因的表达和功能, 从而促进肿瘤的发生发展。NF- κ B、IL-6、STAT3之间存在着相互联系, IL-1、肿瘤坏死因子 α (TNF- α)等细胞因子可通过上调NF- κ B的活性促进IL-6的分泌, 从而间接地活化STAT3。STAT3可通过促进细胞的增殖、迁移及肿瘤细胞的上皮-间质转化等促进肿瘤的进展。未来需要进一步验证NF- κ B/IL-6/STAT3分子通路在MWCNTs致胸膜间皮细胞恶性转化中的作用。

综上所述, MWCNTs可促进胸膜间皮细胞的恶性转化, 且巨噬细胞产生的炎症反应可增强MWCNTs致胸膜间皮细胞恶性转化的能力。在此过程中可能存在着NF- κ B/IL-6/STAT3分子通路的激活, 但需要进一步的实验加以验证。

参考文献

- [1] ADEL AM, ABDEL HAFEEZ ZM, EL SHEIKH ET, et al. Malignant pleural mesothelioma: a retrospective analysis of clinicopathological and survival data[J]. Thorac Cancer, 2011, 2(1): 16-23.
- [2] ROBINSON BW, MUSK AW, LAKE RA. Malignant mesothelioma[J]. Lancet, 2005, 366(9483): 397-408.
- [3] BEHABTU N, YOUNG CC, TSENTALOVICH DE, et al. Strong, light, multifunctional fibers of carbon nanotubes with ultrahigh conductivity[J]. Science, 2013, 339(6116): 182-186.
- [4] DONALDSON K, POLAND CA, MURPHY FA, et al. Pulmonary toxicity of carbon nanotubes and asbestos—similarities and differences[J]. Adv Drug Deliv Rev, 2013, 65(15): 2078-2086.
- [5] SANCHEZ VC, PIETRUSKA JR, MISELIS NR, et al. Biopersistence and potential adverse health impacts of fibrous nanomaterials: what have we learned from asbestos?[J]. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2009, 1(5): 511-529.
- [6] JI JH, WOO D, LEE SB, et al. Detection and characterization of nanomaterials released in low concentrations during multi-walled carbon nanotube spraying process in a clean room[J]. Inhal Toxicol, 2013, 25(14): 759-765.
- [7] LEE JS, CHOI YC, SHIN JH, et al. Health surveillance study of workers who manufacture multi-walled carbon nanotubes[J]. Nanotoxicology, 2015, 9(6): 802-811.

- [8] NAGAI H, OKAZAKI Y, CHEW SH, et al. Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis[J]. Proc Natl Acad Sci USA, 2011, 108(49): E1330-E1338.
- [9] RITTINGHAUSEN S, HACKBARTH A, CREUTZENBERG O, et al. The carcinogenic effect of various multi-walled carbon nanotubes (MWCNTs) after intraperitoneal injection in rats [J]. Part Fibre Toxicol, 2014, 11: 59.
- [10] XU J, ALEXANDER DB, FUTAKUCHI M, et al. Size- and shape-dependent pleural translocation, deposition, fibrogenesis, and mesothelial proliferation by multiwalled carbon nanotubes[J]. Cancer Sci, 2014, 105(7): 763-769.
- [11] MAGDOLENOVA Z, COLLINS A, KUMAR A, et al. Mechanisms of genotoxicity. A review of *in vitro* and *in vivo* studies with engineered nanoparticles[J]. Nanotoxicology, 2014, 8(3): 233-278.
- [12] DONALDSON K, POLAND CA, SCHINS RP. Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies[J]. Nanotoxicology, 2010, 4(4): 414-420.
- [13] LUANPITPONG S, WANG L, DAVIDSON DC, et al. Carcinogenic potential of high aspect ratio carbon nanomaterials [J]. Environ Sci Nano, 2016, 3(3): 483-493.
- [14] MURPHY FA, SCHINWALD A, POLAND CA, et al. The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells[J]. Part Fibre Toxicol, 2012, 9: 8.
- [15] LIANG J, NAGAHASHI M, KIM EY, et al. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer [J]. Cancer Cell, 2013, 23(1): 107-120.
- [16] PYNE NJ, PYNE S. Sphingosine 1-phosphate is a missing link between chronic inflammation and colon cancer[J]. Cancer Cell, 2013, 23(1): 5-7.
- [17] LOHCHAROENKAL W, WANG L, STUECKLE TA, et al. Chronic exposure to carbon nanotubes induces invasion of human mesothelial cells through matrix metalloproteinase-2 [J]. ACS Nano, 2013, 7(9): 7711-7723.
- [18] DONALDSON K, MURPHY FA, DUFFIN R, et al. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma[J]. Part Fibre Toxicol, 2010, 7: 5.
- [19] SCHINWALD A, CHERNOVA T, DONALDSON K. Use of silver nanowires to determine thresholds for fibre length-dependent pulmonary inflammation and inhibition of macrophage migration *in vitro*[J]. Part Fibre Toxicol, 2012, 9: 47.
- [20] BROWN DM, KINLOCH IA, BANGERT U, et al. An *in vitro* study of the potential of carbon nanotubes and nanofibres to induce inflammatory mediators and frustrated phagocytosis [J]. Carbon, 2007, 45(9): 1743-1756.
- [21] XIE TX, WEI D, LIU M, et al. Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis[J]. Oncogene, 2004, 23(20): 3550-3560.

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