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研究报告

ω-3 多不饱和脂肪酸抗肿瘤新途径——抑制具核梭杆菌黏附宿主细胞

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摘 要:【背景】大量文献报道 ω-3 多不饱和脂肪酸尤其是二十二碳六烯酸(Docosahexaenoic Acid，DHA)与二十碳五烯酸(Eicosapentaenoic Acid，EPA)具有抗肿瘤作用，但是其抗肿瘤机制还不够完善。【目的】探究 ω-3 多不饱和脂肪酸、具核梭杆菌以及结直肠癌三者之间的关联。【方法】在检测二十二碳六烯酸、二十碳五烯酸、α-亚麻酸(α-Linolenic Acid，ALA)等 ω-3 多不饱和脂肪酸对人结直肠腺癌细胞 Caco-2、正常结肠上皮细胞 NCM460 生长影响的基础上，检测 DHA 等 3 种多不饱和脂肪酸对具核梭杆菌黏附人体细胞以及 *Fap2*、*FadA*、*RadD* 等具核梭杆菌毒力关键基因表达的影响。【结果】30 μg/mL 的 DHA、EPA、ALA 对 Caco-2 生长抑制分别为 9.09%、4.95%、7.52%，而对 NCM460 生长抑制达 31.15%、25.48%、29.11%，而且相关抑制作用仅具有浓度依赖性而无时间依赖性。经 30 μg/mL 的 DHA、EPA、ALA 预处理的具核梭杆菌黏附 Caco-2 细胞的能力分别下降 81.04% (*P*=0)、93.63% (*P*=0)和 68.63% (*P*=0)；而共培养时加入 DHA、EPA、ALA 对具核梭杆菌黏附 Caco-2 细胞的能力没有显著影响。同时，30 μg/mL DHA 处理导致 *F. nucleatum* 的 *Fap2* 基因显著下降 10.22% (*P*=0.027)；30 μg/mL EPA 处理导致 *FadA*、*Fap2* 基因分别显著下降 23.49% (*P*=0)、15.09% (*P*=0.003)；30 μg/mL ALA 处理导致 *FadA* 基因显著下降 26.75% (*P*=0.012)。【结论】综合上述实验结果以及 DHA、EPA、ALA 仅能短时间抑制具核梭杆菌生长等文献报道，我们认为，DHA、EPA 等 ω-3 多不饱和脂肪酸并非简单地直接杀伤或抑制肿瘤细胞和 *F. nucleatum*；抑制 *FadA*、*Fap2* 等黏附相关基因表达，降低 *F. nucleatum* 黏附宿主细胞能力是其抗肿瘤作用的关键组成部分。ω-3 多不饱和脂肪酸等活性物质对 *F. nucleatum* 等在结直肠肿瘤发生、发展中发挥重要作用的肠道细菌的影响与机制应深入开展研究。

关键词: ω-3 多不饱和脂肪酸，具核梭杆菌，结直肠癌，黏附

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New anti-tumor mechanism of ω-3 polyunsaturated fatty acids — inhibiting *Fusobacterium nucleatum* adherence to host cells

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Abstract: [Background] The antitumor mechanism of ω-3 polyunsaturated fatty acids represented by DHA and EPA has not been fully investigated. **[Objective]** To explore the relationship among ω-3 polyunsaturated fatty acids, *Fusobacterium nucleatum* and colorectal cancer. **[Methods]** After the suppressive effect of ω-3 polyunsaturated fatty acids (DHA, EPA, ALA) on human colon cancer cell line, Caco-2, and normal colon epithelium cell line, NCM460, was assayed, we investigated the impact of these ω-3 polyunsaturated fatty acids on *F. nucleatum*, including growth, adhesive ability to Caco-2 cells, and the expression of virulence genes such as *Fap2*, *FadA* and *RadD*. **[Results]** After treated with 30 μg/mL DHA, EPA or ALA respectively, the growth of Caco-2 cells were suppressed 9.09%, 4.95% and 7.52% correspondingly, meanwhile the growth of NCM460 cells were suppressed 31.15%, 25.48%, 29.11%, and only dose-dependent effect was identified. After treated with 30 μg/mL DHA, EPA and ALA for 12 hours, the adhesive ability of *F. nucleatum* to Caco-2 cells was inhibited by 81.04% (P=0), 93.63% (P=0) and 68.63% (P=0) respectively, which was consistent with the transcriptive level assay results of *FadA*, *Fap2* in *F. nucleatum*. The expression of *Fap2* was considerably suppressed by 10.22% (P=0.027) after 30 μg/mL DHA treatment; *FadA*, *Fap2* were markedly suppressed by 23.49% (P=0) and 15.09% (P=0.003) by 30 μg/mL EPA; and for 30 μg/mL ALA treatment, *FadA* was significantly suppressed by 26.75% (P=0.012). **[Conclusion]** Based on our above results and previous reports that DHA, EPA and ALA only inhibited the growth of *F. nucleatum* temporally, we proposed that ω-3 polyunsaturated fatty acids i-e EPA and DHA significantly attenuated the adhesive ability of *F. nucleatum* to host cells via suppressing the expression of adhesion-related genes such as *FadA*, *Fap2*, which made major contribution to their antitumor activity, rather than inhibiting the growth of tumor cells and *F. nucleatum* directly. The effects and mechanisms of ω-3 polyunsaturated fatty acids i-e DHA and EPA on gut bacterium i-e *F. nucleatum* that play important roles in the initiation and progression of colorectal tumors deserve further research.

Keywords: ω-3 polyunsaturated fatty acids, *Fusobacterium nucleatum*, colorectal cancer, adhesion

许多体内外以及临床研究表明，二十二碳六烯酸(Docosahexaenoic Acid，DHA)、二十碳五烯酸(Eicosapentaenoic Acid，EPA)等 ω-3 多不饱和脂肪酸具有显著的预防及治疗肿瘤发生和发展的作用[1-4]。如喂食富含 DHA 食物的神经母细胞瘤移植瘤大鼠肿瘤生长速度显著减慢[5]；补充 DHA 的乳腺癌化疗患者中，血浆、红细胞中 DHA 水平高的患者相对于含量低的患者，肿瘤进展延迟和总生存期延长[6]。结直肠癌[7]、胃肠癌[8]、乳腺癌[9]等多种肿瘤研究均表明 ω-3 多不饱和脂肪酸通过增加细胞膜流动性[10]、抗炎活性[11]、减少促进致癌物前列腺素 E2 的合成[7]、增加细胞内 ROS 水平[12]等途径抑制肿瘤细胞生长[13]、诱导肿瘤细胞凋亡[13]。然而，Hanson 等 2020 年针对 108 194 人的研究结果显示，补充 DHA、EPA、ALA 对肿瘤发生以及肿瘤患者死亡风险几乎没有影响[14]。如此矛盾的相关研究报道揭示不饱和脂肪酸对肿瘤发生、发展作用的复杂性，暗示可能存在未知途径与作用。

Bray 等 2018 年的研究报道显示，全球结直肠

癌发病率和死亡率分别位于恶性肿瘤的第 3 位与第 2 位[15]。近年来肠道微生物菌群功能或结构失调与结直肠癌之间存在密切关联的研究报道越来越多[16-20]。其中，Chen 等发现相较于健康志愿者，结直肠癌患者的组织样本中 *Fusobacterium*、*Porphyromonas*、*Peptostreptococcus*、*Gemella*、*Mogibacterium* 和 *Klebsiella* 等属菌的丰度显著增加，同时，*Feacalibacterium*、*Blautia*、*Lachnospira*、*Bifidobacterium*和 *Anaerostipes* 等属菌丰度显著减少[21]。

近年来，对革兰氏阴性专性厌氧的具核梭杆菌(*F. nucleatum*)与结直肠癌(Colorectal Cancer，CRC)等肿瘤发生、发展[22]、化疗耐药等密切相关[23-25]，引起了国内外专家的广泛关注。研究表明，*F. nucleatum* 菌体[26-29]、表面蛋白[30-37]及脂多糖[38]等代谢物分别具有促进肿瘤细胞侵袭、诱发炎症反应、促进细胞生长分裂、免疫细胞招募等多样、全局的作用[39]。

F. nucleatum 表面蛋白 FadA 与结直肠癌细胞表面的 E-钙黏蛋白结合能激活 β-Catenin 信号通路，促进肿瘤细胞的增殖[22]。Fap2 作为 *F. nucleatum* 与人体细胞结合的另一个关键表面蛋白，通过与 CRC 细胞过表达的 Gal-GalNAc 结合，导致 *F. nucleatum* 在 CRC 病灶部位富集[35]。Fap2 蛋白同时还能与 T 淋巴细胞和 NK 细胞表面的抑制性受体 TIGIT 作用，导致 NK 细胞毒性和淋巴细胞活性的抑制[40]，最终保护 CRC 细胞免受自然杀伤细胞(Natural Killer Cell，NK)毒性、肿瘤浸润淋巴细胞和 T 细胞攻击。RadD 作为 *F. nucleatum* 的另一个重要黏附素通过与 *Streptococcus mutans*[41]、*S. mutans*、白色念珠菌[42]的结合，促进口腔细菌聚集及生物膜的形成。由此可见，*F. nucleatum* 很可能是人体异常微生物群落形成的先锋物种。

基于上述研究报道，尤其是 DHA 与 EPA 能够下调 *F. nucleatum FadA* 表达[43]的研究报道，本研究计划通过系统分析 DHA、EPA、ALA 对具核梭杆菌、结直肠正常与肿瘤细胞的生长以及对具核梭杆菌与人体细胞互作的影响，探究多不饱和脂肪酸、*F. nucleatum*、结直肠肿瘤三者的相互关系，为明确多不饱和脂肪酸抑制肿瘤机制提供信息。

1 材料与方法

1.1 材料

1.1.1 细胞株与细胞培养

细胞株：人结直肠腺癌细胞 Caco-2 和正常结肠上皮细胞 NCM460 由本实验室保存。

细胞培养：Caco-2 细胞采用含 20%胎牛血清的 DMEM 高糖培养基培养；NCM460 细胞采用含 10%胎牛血清的 DMEM 高糖培养基培养；2 种细胞于 37 °C、5% CO_2、饱和湿度的细胞培养箱中培养。细胞传代、冻存等操作按常规方法进行。

1.1.2 实验菌株与培养

实验菌株：*F. nucleatum* ATCC 25586 由本实验室保存。

菌株培养：*F. nucleatum* 采用 BHI 培养基在 85% N_2、5% CO_2、10% H_2、37 °C 厌氧培养。

1.1.3 主要试剂和仪器

DMEM 高糖培养基，HyClone 公司；胎牛血清，BI 公司；DHA、EPA、ALA，上海阿拉丁生化科技股份有限公司；MTT 细胞增殖及细胞毒性检测试剂盒，南京凯基生物科技发展有限公司；细菌总 RNA 提取试剂盒，Magen 生物公司；FastQuant cDNA 第一链合成试剂盒，天根生化科技(北京)有限公司；Hieff UNICON® qPCR SYBR Green Master Mix，上海翊圣生物科技有限公司。多功能酶标仪，Tecan 公司；荧光定量 PCR 仪，Thermo Fisher 公司；荧光正置显微镜，蔡司公司。

1.2 方法

1.2.1 DHA、EPA、ALA 的细胞毒性检测

采用 MTT 方法检测了 DHA、EPA、ALA 的细胞毒性。将 Caco-2 细胞、NCM460 细胞培养至 80%覆盖时，胰酶消化，96 孔板每孔接种 5 000 个细胞。待细胞过夜贴壁后，加入 DHA、EPA、ALA 使终浓度分别为 0、30、60、120 μg/mL，处

理 24、48、72 h 后，采用 MTT 细胞增殖及细胞毒性检测试剂盒进行细胞活性检测。

1.2.2 具核梭杆菌对结直肠腺癌细胞的黏附试验

F. nucleatum 用 ω-3 多不饱和脂肪酸预处理组：将培养至指数末期的 *F. nucleatum* 按 1%比例分别接种于含 30 μg/mL DHA、EPA、ALA 的 BHI 培养基继续培养 12 h 后，将菌悬液 4 000 r/min 离心 10 min。用 DMEM 培养基悬浮细菌，调整菌浓度至 1×10^8 CFU/mL。

在加入细胞培养级盖玻片的 24 孔细胞培养板中铺 1×10^5 个 Caco-2 细胞，置 37 °C、5% CO_2 培养箱中培养至 80%覆盖度时，用 PBS 洗 3 遍，每孔分别加入经 DHA、EPA、ALA 预处理或未处理的 500 μL 1×10^8 CFU/mL 具核梭杆菌。

共培养组：此时未处理组中加入终浓度为 30 μg/mL 的 DHA、EPA、ALA。对照组与各实验组均设置 3 个复孔。两组分别继续于 37 °C、5% CO_2 条件下培养 2 h 后，吸去培养液，用 PBS 冲洗 3 遍，洗去未结合的具核梭杆菌，自然干燥，冰甲醇固定 10 min，革兰氏染色，100 倍油镜观察、拍照、计单细胞黏附的细菌数。

1.2.3 总 RNA 提取、反转录、定量 PCR 检测

待 *F. nucleatum* 长到对数生长期，即 OD_{600} 为 0.5 左右时分别用 30 μg/mL 的 DHA、EPA、ALA 处理 3 h，随后采用细菌总 RNA 提取试剂盒提取具核梭杆菌总 RNA，具体操作按试剂盒说明书进行。反转录根据 FastQuant cDNA 第一链合成试剂盒进行反转录，总 RNA 使用量为 1 μg。定量 PCR 采用 Hieff UNICON® qPCR SYBR Green Master Mix 配制 20 μL 的反应体系进行实验，操作按说明书进行。*rpoB* 等定量 PCR 引物采用 Primer 3 网站设计，由捷瑞公司合成，引物具体序列见表 1。定量 PCR 条件为：95 °C 15 min；95 °C 10 s，60 °C 20 s，72 °C 20 s，共 40 个循环，最后进行溶解曲线分析。基因表达相对定量采用 $2^{-\Delta\Delta C_t}$ 方法计算，内参基因为 *rpoB*。

1.3 统计学分析

实验结果组间差异采用 SPSS 25.0 软件进行 ANOVA 分析，当 $P\leq0.05$ 时认为差异具有统计学意义。

2 结果与分析

2.1 DHA、EPA、ALA 对 Caco-2、NCM460 的细胞毒性作用

用不同浓度的 DHA、EPA、ALA 分别处理正常结肠上皮细胞 NCM460 24、48 和 72 h，结果如图 1 所示，30 μg/mL DHA 处理的各时间处理组 NCM460 相对存活率分别为 60.19%、72.00%和 74.35%；60 μg/mL DHA 处理组分别是 3.52%、3.81%和 2.76%；120 μg/mL DHA 处理组分别是 6.24%、3.16%和 2.76%。30 μg/mL EPA 处理组分别是 67.22%、85.41%和 70.94%；60 μg/mL EPA 处理组分别是 9.04%、4.35%和 4.42%；120 μg/mL EPA 处理组分别是 6.65%、3.55%和 2.25%。

表 1 本研究涉及的引物

Table 1 Primers designed for this study

基因 Gene	引物 Primers	序列 Sequences (5′→3′)
Fap2	正向 Forward	CAGTTGCTACAGATGGTGTGGTAGG
	反向 Reverse	TGAATTTGCTTCTCCATACATTCCTG
FadA	正向 Forward	GCAGCAAGTTTAGTAGGTGAATTACAA
	反向 Reverse	GTCTAGCAGCGTCAGCTTGTG
RadD	正向 Forward	GATATGAGCAACAACAATGCGAAAG
	反向 Reverse	GTGCCTTGACCATCAGAAGAAACTT
rpoB	正向 Forward	ATCTTCCACCATCAAGTTGAACCAT
	反向 Reverse	GCTTCTGGAAATAGACCTGAATGGA

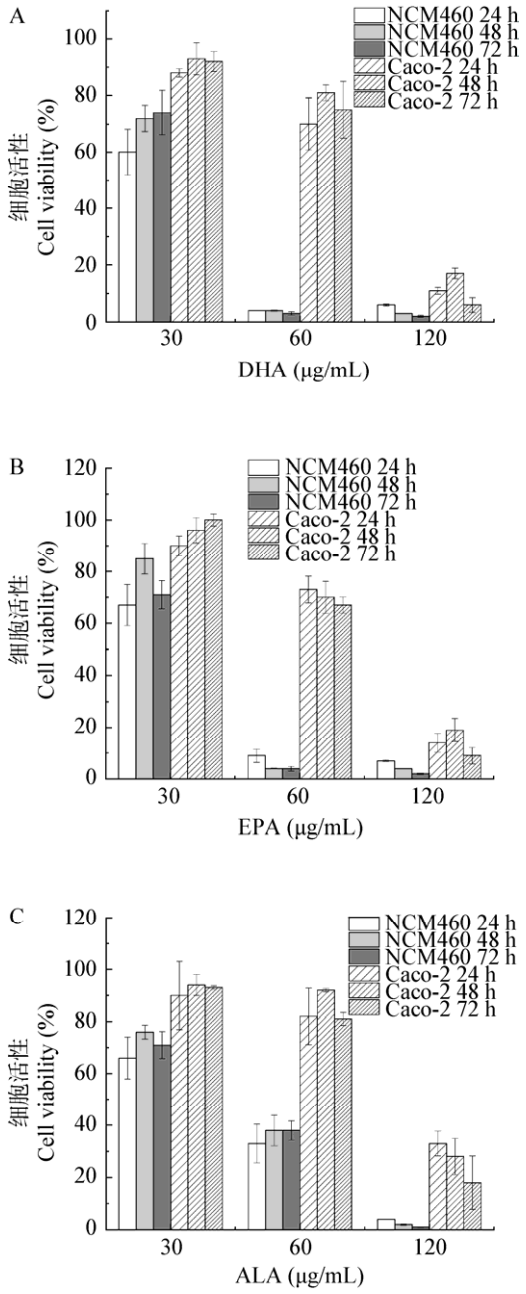

图 1 DHA (A)、EPA (B)、ALA (C)对 Caco-2、NCM460 细胞的毒性作用

Figure 1 Cytotoxicity evaluation of DHA (A), EPA (B) and ALA (C) on Caco-2, NCM460 cells

30 μg/mL ALA 处理组分别是 65.73%、75.84%和 71.09%；60 μg/mL ALA 处理组分别是 32.77%、38.24%和 37.93%；120 μg/mL ALA 处理组分别是 3.97%、1.87%和 1.42%。30、60、120 μg/mL 各浓度的不同时间处理组之间差异不显著。

对于结直肠肿瘤细胞 Caco-2，30 μg/mL DHA 处理的各时间处理组相对存活率分别是 87.71%、93.25%和 91.76%；60 μg/mL DHA 处理组分别是 69.62%、80.75%和 74.51%；120 μg/mL DHA 处理组分别是 11.04%、17.20%和 5.51%。30 μg/mL EPA 处理组分别是 89.87%、95.74%和 99.54%；60 μg/mL EPA 处理组分别是 72.83%、69.78%和 67.18%；120 μg/mL EPA 处理组分别是 13.81%、19.49%和9.34%。30 μg/mL ALA 处理组分别是 89.91%、94.04%和 93.49%；60 μg/mL ALA 处理组分别是 82.39%、92.07%和 80.54%；120 μg/mL ALA 处理组分别是 33.16%、28.21%和 17.83%。30、60、120 μg/mL 各浓度的不同时间处理组之间差异不显著(图 1)。

2.2 DHA、EPA、ALA 对 *F. nucleatum* 黏附能力的影响

我们前期分别用 30、60、120 μg/mL 等不同浓度的 DHA、EPA、ALA 处理 *F. nucleatum* 0−24 h，经具核梭杆菌生长检测发现，前 14 h DHA、EPA、ALA 抑制率较强，最高抑制率分别是 71.61%、77.38%、81.91%；但随着培养时间的延长，抑制作用逐渐减弱，处理 24 h 后最高抑制率分别下降至 16.83%、14.99%、17.24% (结果未展示)。该结果与文献[43]报道一致。

综合考虑 DHA、EPA、ALA 对 Caco-2、NCM460 和 *F. nucleatum* 的生长抑制作用，后续选择 30 μg/mL 浓度的 DHA、EPA、ALA 处理 *F. nucleatum* 并检测其黏附 Caco-2 能力的变化。

如图 2 所示，经 30 μg/mL DHA、EPA、ALA 处理 12 h 的 *F. nucleatum*，Caco-2 细胞上黏附的细菌数量分别比对照组减少了 81.04% (P=0)、93.63% (P=0)和 68.63% (P=0)。

当 Caco-2 与 *F. nucleatum* 共培养时加 30 μg/mL DHA、EPA、ALA，如图 3 所示，Caco-2 细胞上黏附的细菌数量未发生显著变化。

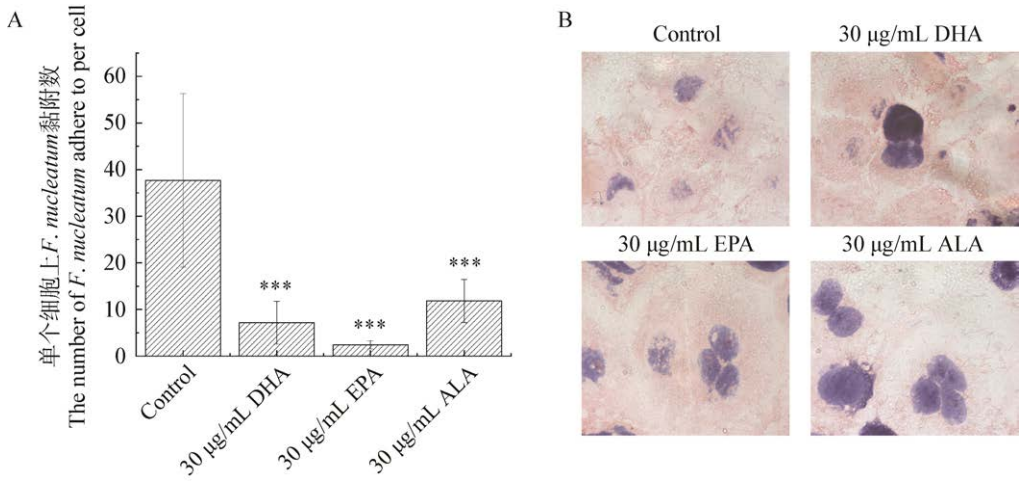

图 2　30 μg/mL DHA、EPA、ALA 预处理抑制 *F. nucleatum* 黏附 Caco-2 细胞

Figure 2　30 μg/mL DHA, EPA and ALA pretreatment suppress the adhesive ability of *F. nucleatum* to Caco-2 cells

注：A：单个细胞上 *F. nucleatum* 平均黏附数柱状图；B：*F. nucleatum* 黏附照片。***：P<0.001

Note: A: Column chart of the average number of *F. nucleatum* adhere to single cell; B: Pictures of *F. nucleatum* adhesion. ***: P<0.001

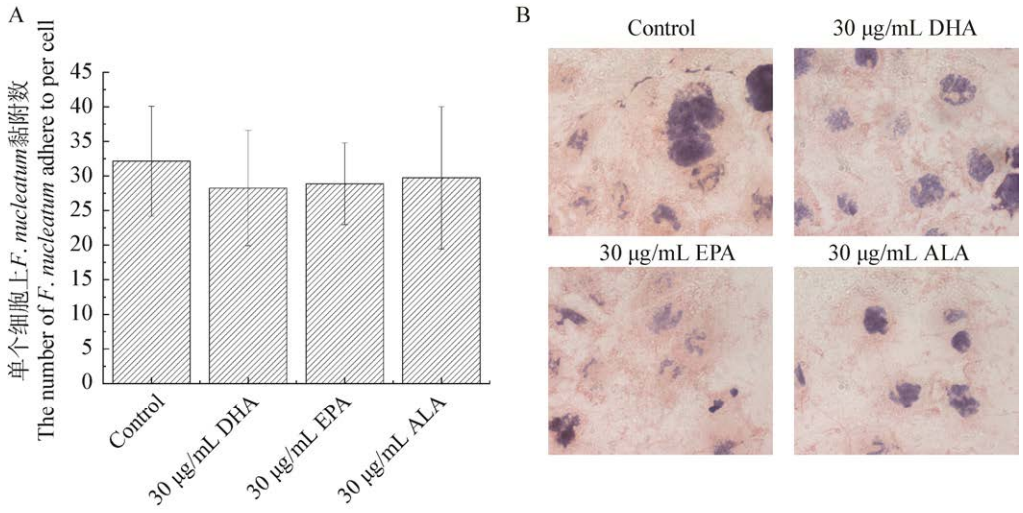

图 3　30 μg/mL DHA、EPA、ALA 直接作用对 *F. nucleatum* 黏附 Caco-2 细胞没有影响

Figure 3　Treating with 30 μg/mL DHA, EPA and ALA directly have no influence on the adhesive ability of *F. nucleatum* to Caco-2 cells

注：A：单个细胞上 *F. nucleatum* 平均黏附数柱状图；B：*F. nucleatum* 黏附照片

Note: A: Column chart of the average number of *F. nucleatum* adhere to single cell; B: Pictures of *F. nucleatum* adhesion

2.3　DHA、EPA、ALA 对 *F. nucleatum Fap2*、*FadA*、*RadD* 基因表达水平的影响

检测 30 μg/mL 的 DHA、EPA、ALA 处理 3 h 的具核梭杆菌 *FadA*、*Fap2* 等黏附相关基因、定殖有关基因 *RadD* 的表达量发现，DHA 处理组的 *Fap2* 基因表达比对照组下调了 10.22% (P=0.027)，与对照组有显著性差异(P<0.05)。EPA 处理组的 *FadA*、*Fap2* 基因表达分别显著下调了 23.49% (P=0)、15.09% (P=0.003)。ALA 处理组的 *FadA* 基因表达比对照组显著下调了 26.75% (P=0.012)，详见图 4。

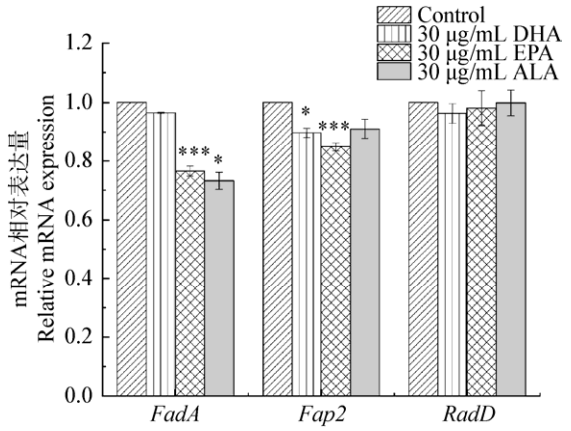

图 4　30 μg/mL DHA、EPA、ALA 对 *Fap2*、*FadA*、*RadD* 表达量的影响

Figure 4　Effects of 30 μg/mL DHA, EPA and ALA on the expression of *Fap2*, *FadA*, *RadD*

Note: *: $P<0.05$; ***: $P<0.001$

3　讨论与结论

许多动物实验、流行病学等临床研究表明 ω-3 多不饱和脂肪酸具有降低结直肠癌风险的作用[7,13,44]，但也有无效的报道[14]。这种研究结果不一致甚至矛盾的现象，反映了 ω-3 多不饱和脂肪酸抗肿瘤作用机制的复杂性。如表 2 所示，不同细胞对不同种类的 ω-3 多不饱和脂肪酸敏感性各不相同。如：66 μg/mL DHA 处理 24 h 时，人牙龈成纤维细胞(hGFs)活性下降约 50%，而人牙周膜细胞(hPDLCs)仅下降约 30%；同时，60 μg/mL 的 EPA 对此 2 种牙周细胞活性基本没有影响[43]。然而即使浓度高达 197 μg/mL 的 DHA 处理 24 h，大鼠胶质母细胞瘤细胞 C6 细胞活性仍保持 80%左右[45]。然而仅用 20 μg/mL 和 13 μg/mL DHA 处理人胃癌细胞 GT-38[46]与人膀胱癌细胞 EJ-138[47] 48 h，就能使 2 种细胞活性下降约 50%。

Toit-Kohn 等采用低血清培养基培养 Caco-2、NCM460 细胞时，16.4 μg/mL DHA 能够显著降低 Caco-2 细胞活力达 80%，而正常结肠细胞 NCM460 的活力不受影响[49-50]。本研究中，采用正常培养条件培养 Caco-2、NCM460 细胞时，30 μg/mL 的 DHA、EPA、ALA 处理 Caco-2 细胞活性仅下降 10%左右，而 NCM460 细胞活性则下降 30%左右。即使是抑制作用最弱的 ALA，仍呈现抑制正常细胞(NCM460)作用强于肿瘤细胞(Caco-2)的类似现象，详见图 1。同时，研究结果显示，DHA、

表 2　DHA、EPA 对不同种类细胞的抑制作用

Table 2　Suppressive effect of DHA and EPA on different kinds of cells

细胞种类 Cell lines or kinds	处理方法 Experimental method	细胞活性 Cell viability (%)	参考文献 References
人牙龈成纤维细胞 Human gingival fibroblasts (hGFs)	66 μg/mL DHA 24 h 60 μg/mL EPA 24 h	About 50% 细胞毒性不明显 No obvious cytotoxicity	[43]
人牙周膜细胞 Human periodontal ligament cells (hPDLCs)	66 μg/mL DHA 24 h 60 μg/mL EPA 24 h	About 70% 细胞毒性不明显 No obvious cytotoxicity	[43]
大鼠胶质母细胞瘤细胞 C6 Rat glioblastoma cell line C6	197 μg/mL DHA 24 h	About 80%	[45]
人胃癌细胞 GT-38 Epstein-Barr virus-associated gastric carcinoma GT38	20 μg/mL DHA 48 h	About 50%	[46]
人膀胱癌细胞 EJ-138 与 HTB-9 Human bladder cancer cell lines EJ-138 and HTB-9	13 μg/mL DHA 48 h	About 50%	[47]
大鼠胶质细胞 C6G Rat glioma cells C6G	33 μg/mL DHA 24 h	45.2%	[48]
人神经母细胞瘤细胞 SH-SY5Y Neuroblastoma cell SH-SY5Y	33 μg/mL DHA 24 h	45.9%	[48]
星形胶质细胞 Astrocytes	33 μg/mL DHA 24 h	100%	[48]

EPA、ALA 对 Caco-2、NCM460 的生长抑制作用仅具有显著的剂量依赖作用，无时间依赖作用。

鉴于上述实验结果，我们认为 DHA、EPA、ALA 的抗肿瘤作用不仅仅是由于直接抑制肿瘤细胞生长，而是通过其他间接途径实现。同时，文献报道与本研究预实验发现 DHA、EPA、ALA 仅能在 14 h 内短暂抑制 *F. nucleatum* 生长[43,51-52]，之后抑制作用基本消失。该实验结果表明，DHA、EPA、ALA 也不是通过杀死或完全抑制 *F. nucleatum* 的生长发挥抗肿瘤作用。

进一步检测 DHA、EPA、ALA 对 *FadA*、*Fap2*、*RadD* 等具核梭杆菌黏附、侵袭肠上皮细胞过程关键基因[39]表达水平的影响以及对 *F. nucleatum* 黏附宿主细胞能力的影响等实验发现，30 μg/mL DHA、EPA、ALA 预处理能够显著降低 Caco-2 细胞上附着的具核梭杆菌数量，降幅分别达到 81.04% (P=0)、93.63% (P=0) 和 68.63% (P=0)。该结果与文献报道的 DHA 和 EPA 抗肿瘤活性强于 ALA 报道一致[53]。但 *F. nucleatum* 与 Caco-2 共培养时再加入相同浓度的 DHA、EPA、ALA 对 *F. nucleatum* 黏附能力没有显著影响，该实验结果进一步表明 DHA、EPA、ALA 并不直接影响 *F. nucleatum* 对肿瘤细胞的黏附能力。

根据 DHA、EPA、ALA 处理分别显著下调 *F. nucleatum* 的 *Fap2*、*FadA* 表达的实验结果，我们认为，抑制 *FadA*、*Fap2* 等黏附相关基因表达减弱了 *F. nucleatum* 黏附宿主细胞的能力，是 ω-3 多不饱和脂肪酸抗肿瘤活性的关键组成部分，相关分子机制值得深入开展研究。

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