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# Research Advances of AMMECR1

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## Abstract

**AMMECR1 (Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis chromosomal region gene 1) is a gene from the novel X-linked contiguous gene deletion syndrome AMME critical region. It encodes a transcript that is conserved throughout the course of evolution. There is a considerable degree of homology between the AMMECR1 proteins from different species ranging from bacteria and archaea to eukaryotes. This conservation suggests that AMMECR1 and its homologue proteins may exert essential functions in a variety of organisms. In this review, we will describe that AMMECR1 expression, crystal structure, phosphorylation, function-related proteins, miRNAs targeting to AMMECR1 and its interaction partner to promote the study of AMMECR1.**

## Keywords

**AMMECR1, Phosphorylation, Related Proteins, miRNA**

# AMMECR1的研究进展

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## 摘　要

**AMMECR1是X-连锁邻近基因缺陷综合症AMME关键区域基因之一。它是一个非常古老而且保守的基因，**

**从古细菌，细菌，酵母、线虫、果蝇到哺乳类和人，这一蛋白都有很高的同源性，应该在基本的生物学过程中执行某个重要的功能。但AMMECR1的生物学功能研究还很匮乏。本文从AMMECR1的表达、同源蛋白的晶体结构、磷酸化、功能相关蛋白、靶向AMMECR1的miRNA、AMMECR1可能的相互作用蛋白等方面进行综述，为开展AMMECR1研究的提供参考。**

## 关键词

**AMMECR1，磷酸化，相关蛋白，miRNA**

## 1. 引言

AMMECR1 (Alport syndrome, mental retardation, midface hypoplasia, elliptosis chromosomal region gene 1)是 X-连锁邻近基因缺陷综合症(contiguous gene deletion syndrome) AMME 关键区域基因之一[1]。AMMECR1 是一个非常古老而且保守的基因，从古细菌，细菌，酵母、线虫、果蝇到哺乳类和人，这一蛋白都有很高的同源性，可以想见，它一定在细胞生命活动的核心过程中发挥作用。目前比较明确的是该区域内编码Ⅳ型胶原α-5 链基因 COL4A5 点突变或基因内删除将引起血管性球性肾炎。然而带有不包含 COL4A5 基因但含有包括 AMMECR1 在内的 9 个基因的 X 染色体删除的患者，仍表现中等智力障碍(moderate intellectual disability)，神经性听力损失(sensorineural hearing loss)，面部发育不全(facial dysmorphism)，幽门狭窄(pyloric stenosis)以及肠梗阻(intestinal obstruction) [2]。不过，AMMECR1 是否和这些病理现象直接相关有待进一步研究。

AMMECR1 有 6 个外显子，产生 4 种拼接形式转录子(transcripts)，最长的转录子编码 333 个氨基酸的多肽，是 AMMECR1 的常见形式；位居第二的转录子缺失了第二外显子(氨基酸 159-195)；如果翻译从 mRNA 5′端第二个甲硫氨酸密码子开始则形成缺少 N 端 123 个氨基酸(氨基酸 1-123)的多肽；第一外显子和其下游内含子中一个额外的外显子(外显子 2′)拼接则形成仅含 N 端 157 个氨基酸的多肽[1]。AMMECR1 的生物学功能研究是极其匮乏的。本文探讨 AMMECR1 研究现状及可能参与的生物学过程。

## 2. AMMECR1 的表达

本 AMMECR1 组织分布一般比较广泛，但还能在一些特殊细胞或条件下表达、不表达甚至被删除(表 1)。如在兔白内障手术后水状液(aqueous humor，AH)样品中以及在养殖于含乙炔雌二醇(ethinylestradiol)水体达 14 天的鱼类精巢组织中均有表达[3] [4]；但在鼠滋养层干细胞 K4GFP 中，AMMECR1 不表达[5]；而在智力障碍者的外周血白细胞及人类不同恶性程度的眼色素层黑色素瘤(*Uveal melanoma*)细胞，AMMECR1 是染色体删除的[2] [6]。如果 AMMECR1 酵母(*S. pombe*)同源物 Spac688.03c 删除、酵母细胞对紫外线中等敏感[7]，因此，它可能和 DNA 的修复有关。

## 3. AMMECR1 同源蛋白的晶体结构

Pyrococcus horikoshii OT3 蛋白 PH0010 和人 AMMECR1 C-端氨基酸序列类似， PH0010 晶体结构显示它有大小两个结构域，两个结构域间有一个可变的裂隙[8]，结构比较分析显示，它含有一个 RAGNYA (Ribosomal proteins L3 and L1, ATP grasp modules, the GYF domain, DNA recombination proteins of the NinB family from caudate bacteriophages, the C-terminal DNA-interacting domain of the Y-family DNA polymerases, the uncharacterized enzyme AMMECR1)折叠，像 tRNA Wybutosine 生物合成酶 Tyw3p, AMMECR1 可能催化某个 RNA 的碱基修饰[9]。

**Table 1.** AMMECR1 expression
**表 1.** AMMECR1 的表达

| 物种 | 细胞或组织 | 条件 | mRNA | 参考文献 |
|---|---|---|---|---|
| 兔 | AH 手术样品 | | 表达 | [3] |
| 青鳉 | 精巢 | 含乙炔雌二醇水体养殖 14 天 | 表达 | [4] |
| 鼠 | 滋养层干细胞 $K4^{GFP}$ | H3K27me3 甲基化减少 | 不表达 | [5] |
| 人 | 外周血白细胞 | 智力障碍者 | 删除 | [2] |
| 人 | 眼色素层黑色素瘤细胞 | 易发 MM、MS、DM | 删除 | [6] |
| 酵母 | Spac688.03c 基因删除 | UV 中等敏感 | 删除 | [7] |

## 4. AMMECR1 磷酸化

相对于其它 AMMECR1 同源物，人 AMMECR1N 端多出一段富含甘氨酸和丝氨酸的多肽。生物信息学分析显示 AMMECR1 有 6 个 PKC (protein kinase C)磷酸化位点(S121、T150、T163、S249、T291、S299)和 3 个酪蛋白激酶(casein kinase II)磷酸化位点(T206、T221、S305)，23 个潜在的 N-豆蔻酰化位点[1]。有趣的是，这些磷酸化位点都不在人 AMMECR1 富含甘氨酸和丝氨酸的 N 端，但质谱结果显示人 AMMECR1 有六个磷酸化位点(S15、S16、S19、S21、S29 和 S34) [10]-[13]，它们都分布在富含甘氨酸和丝氨酸的 N 端，可见，需要实验证实人 AMMECR1 真正的磷酸化位点。

## 5. AMMECR1 功能相关蛋白

AMMECR1 可能参与多个细胞生物过程，受多个蛋白质或其他因子的调控(表 2)。EPS8 (epidermal growth factor receptor pathway substrate 8，能增强细胞繁殖、迁徙及肿瘤发生)在 HN4 细胞(源自头颈部的原发性鳞状细胞癌细胞)中的过表达、转录因子 TFAP2C(涉及乳腺发育、分化及肿瘤发生)在 MCF7 细胞中的过表达，均能上调 AMMECR1 的表达[14] [15]；GBM (glioblastoma multiforme)是普通侵袭性原发性脑瘤，用葡萄多酚 resveratrol (RV)刺激 GBM-$CD133^{+}$细胞，也能上调 AMMECR1 的表达[16]；新生 7 天的小鼠在低氧(75%)中养 5 天，然后在正常空气中养 12 小时，视网膜 AMMECR1 的表达上调[17]。PHF8 (plant homeodomain (PHD) finger-containing proteins 8，是组蛋白 H3K9 去甲基化酶)。在 HeLa 细胞中的低表达(knockdown)，AMMECR1 的表达下调[18]；在 IFN-$\gamma$/TNF-$\alpha$ 联合刺激下，hcMSCs (human bone marrow (BM)-derived clonal mesenchymal stem cells (MSCs))细胞则能大幅下调 AMMECR1 的表达[19]；FGFR4 (fibroblast growth factor receptor 4) 在 HCC1.2. (hepatocellular carcinoma)中的表达及在高风险 MDS (myelodysplastic syndrome)-$CD34^{+}$细胞及红细胞样细胞(Erythroid cells)在添加 BM-SCs (bone marrow stromal cells)细胞培养上清的培养基中培养，AMMECR1 的表达均下调[20]-[22]。在 AML (acute myeloid leukemia)细胞，decitabine 诱导 MLL5 (human trithorax-group (Trx-G) gene)敲除的细胞和具有 CBE (classic bladder exstrophy)的病人的膀胱组织，AMMECR1 的表达显示明显差异[23] [24]。这些蛋白或因子如何调控 AMMECR1 表达，目前还不清楚。AMMECR1 表达变化显示可能参与在此种条件下细胞的某些生物学过程，如 PHF8 删除，导致 X 连锁智力发育迟缓，在 HeLa 细胞中，降低 PHF8 的表达，导致 AMMECR1 明显下调[18]，而在 IFN-$\gamma$/TNF-$\alpha$ 联合刺激下，hcMSCs 细胞 STAT2 大幅上调，抑制 T 细胞繁殖，此时 AMMECR1 的表达大幅下调[19]，AMMECR1 是否和智力发育及 T 细胞繁殖有关，这些或许值得探讨。

## 6. 靶向 AMMECR1 的 miRNA

miRNAs (MicroRNAs)是真核生物中一类大小长约 20~25 个核苷酸、通常结合在 mRNA 的 3′端非编

码区的非编码 RNA，miRNA 参与各种各样的调节途径，包括发育、病毒防御、造血过程、器官形成、细胞增殖和凋亡、脂肪代谢等等生物信息学分析发现，很多 miRNA 可以靶向 AMMECR1 (表 3)，如 has-miR-517a、miR-191、has-miR-375 和 miR-124 [25]-[28]；把 T3BA 细胞(hES (human embryonic stem)-T3 cells)培养在补充 4 ng/ml bFGF (human basic fibroblast growth factor)和 5 ng/ml activin A 干细胞培养基中，miRNA let-7c 表达仅占对照组的 0.06，let-7c 靶向基因 AMMECR1 表达升高 15.68 倍[29]；在 MDA-MB-435 乳腺癌细胞(不表达内源性 integrin$\alpha 6\beta 4$)中过表达 integrin$\alpha 6\beta 4$，miR-92ab 和 miR-99ab/100 family miRNA 表达受抑，但它们的靶基因 AMMECR1 表达小幅上扬[30]；表达 miR-26b 也降低 AMMECR1 表达[31]；adriamycin 抗性的乳腺癌细胞 MCF-7/ADR，降低 lncRNAs (long non-coding RNAs) ARA (adriamycin resistance associated)的表达，则 AMMECR1 表达增加 3 倍以上[32]。

## 7. AMMECR1 相互作用蛋白

在酵母蛋白质相互作用网络中，AMMECR1 存在于 RNA 加工与运输相关蛋白如 Nup114p、Soh1p、Yra1p 和 Jsn1p 复合物中。在小鼠，AMMECR1 与精子发生蛋白 SPATA22、DNALI1、NME3 和 SMOK1 有相互作用[33]，不过 SMOK1 在人却没有同源蛋白。在人细胞，质谱结果显示他和 CLP1、GFER、PPIL4、TSEN54、ZNF703、ELAV1、UBC 等 7 个蛋白可能存在相互作用[34] [35]。

**Table 2.** Proteins and other factors related to AMMECR1 functions
**表 2.** AMMECR1 功能相关的蛋白质及其他因子

| 物种 | 细胞系或组织 | 条件 | mRNA (倍数) | 参考文献 |
|---|---|---|---|---|
| 人 | HN4 | EPS8 过表达 | 1.8 | [14] |
| 人 | MCF-7 | TFAP2C 过表达 | >1.3 | [15] |
| 人 | GBM-CD133$^+$ | RV 刺激 | >2 | [16] |
| 鼠 | 视网膜 | 相对高氧 12 小时 | 1.336 | [17] |
| 人 | HeLa | PHF8 过表达 | −10 | [18] |
| 人 | hcMSCs | IFN-$\gamma$/TNF-$\alpha$ 刺激 | −100 | [19] |
| 人 | HCC-1.2. | FGFR4 过表达 | −3 | [20] |
| 人 | MDS CD34$^+$ | 来自 MDS 患者 | −3 | [21] |
| 人 | erythroid | BM-SC 条件化培养基培养 | −6.29 | [22] |
| 人 | AML Mll5$^{-/-}$ | decitabine-诱导 | 差异表达 | [23] |
| 人 | 膀胱组织 | 来自 CBE 患者 | 差异表达 | [24] |

**Table 3.** miRNAs targeting to AMMECR1
**表 3.** 靶向 AMMECR1 的 miRNAs

| 细胞或组织 | 状态 | miRNA | 参考文献 |
|---|---|---|---|
| 子宫内膜 | | has-miR-517a | [25] |
| 胃癌 | | miR-191 | [26] |
| 胃癌 | | has-miR-375 | [27] |
| | | miR-124 | [28] |
| T3BA | 补充 bFGF 和 Activin 培养 | let-7c(15.68) | [29] |
| MDA-MB-435 | INTEGRIN $\alpha 6\beta 4$ 过表达 | miR-92ab | [30] |
| | | miR-99ab/100 family (1.7) | |
| MCF7 | 雌激素处理 | miR-26b | [31] |
| MCF-7/ADR | ARA 敲低 | IncRNA(ARA) | [32] |

## 8. 结语

从上文的叙述中可以看到，AMMECR1 的研究结果多为生物信息学预测及各种组学研究的数据，我们很难明确指出 AMMECR1 在执行什么生物学功能，毫无疑问，AMMECR1 的功能研究还需要艰苦的大量的基础性实验工作，只有这样，才会逐步揭示 AMMECR1 这一古老而保守的蛋白的生物学功能，它或许对探明人类某方面的疾病有所帮助。

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