Research on Regulation Mechanism of Aquaporin 3 and Its Effects on Skin

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Abstract

Aquaporin 3 (AQP3) plays an important role in the expression of various skin-related diseases such as vitiligo and psoriasis. We aim to explore the regulatory mechanism of AQP3 and the function of AQP3 on the skin. We summarize the distribution of AQP3 in skin and the regulatory mechanism of AQP3, then point out the function of AQP3 on the skin and the development trend of AQP3 as a new target in the field of cosmetic research.

Keywords: Aquaporin 3, Skin, Regulation, Moisturizing, Anti-aging

Introduction

水通道蛋白 (Verkman *et al.*, 2008) 是上世纪90年代发现的一类在细胞膜上与水分子通透性有关的转运蛋白。迄今为止, 共有200余种水通道蛋白在不同物种中被发现, 其中有13种水通道亚型存在于哺乳动物体内, 分别被命名为AQP0-12 (Takata *et al.*, 2004)。根据水通道蛋白转运功能特异性的差异, 将其分为两个亚家族 (Hara-Chikuma & Verkman, 2008; Jung *et al.*, 1994): 其中一个是水选择通道亚家族, 包括AQP1、AQP2、AQP4、AQP5、AQP6、AQP8、和AQP9; 另一个是水-甘油通道亚家族, 包括AQP3、AQP7、AQP10和AQP11。对于AQP11和AQP12, 其生理功能尚不清楚, 但是有研究显示 (Morishita *et al.*, 2004), 可能参与器官形成。

AQPs广泛分布于机体器官及组织细胞中 (Geng & Yang, 2014; Hara-Chikuma *et al.*, 2009; Venero *et al.*, 2001), 尤其与液体分泌和吸收有关的上皮细胞和内皮细胞含量颇

丰, 参与水的分泌、吸收及细胞内外水的平衡。然而, 在皮肤中表达的水通道蛋白只有AQP3 (Verkman, 2011), 它属于水-甘油通道蛋白亚家族。通过调研发现, AQP3与特异性皮炎、银屑病、白癜风、皮肤肿瘤等皮肤疾病的发生密切相关。故主要从AQP3在皮肤中的分布, AQP3的调控机制以及AQP3对人类皮肤的作用三个方面进行综述, 为通过调节AQP3来护肤的相关化妆品的研发提供科学依据。

Composition and distribution of AQP3 in skin

1994年, Ishibashi *et al.* (1994) 发现了AQP3, 其含有279个氨基酸, 相对分子质量约为 29×10^3 。AQP3是一种跨膜蛋白, 最早被报道是在表皮角质形成细胞中表达 (Matsuzaki *et al.*, 2004)。而且通过调研发现 (Silberstein *et al.*, 1999), 其在表皮的分布主要表达于基底层以上角质层以下部分, 其中在基底层的角质形成细胞膜上的表达

最为强烈，随着角质形成细胞的分化和向上移动而逐渐减少，到角质层完全消失。AQP3在人表皮的这种分布与人表皮水分的分布情况是一致的，与角质层和颗粒层交界处的水分梯度相符合（Tang, 2008）。

Regulation mechanism of AQP3

1. Effects of genes, hormones, and cytokines

通过对水通道蛋白结构的分析（Jung *et al.*, 1994）发现AQP3蛋白分子中具有蛋白激酶磷酸化位点，即蛋白激酶A（protein kinase A, PKA）、蛋白激酶C（protein kinase C, PKC）或者蛋白激酶G（protein kinase G, PKG）的同源序列。研究发现（Itoh *et al.*, 2004; Wu *et al.*, 2014），当腺苷酸环化酶（adenylate cyclase, AC）被激活，会导致细胞内环磷酸腺苷（cyclic adenosine monophosphate, cAMP）的含量增加，进一步激活PKA、PKC或者PKG，从而促进AQP3基因的转录。研究发现当激素失衡时，激素通过过氧化物酶体增殖物激活受体 γ （peroxisome proliferators-activated receptor γ , PPAR γ ）-肿瘤坏死因子 α （tumor necrosis factor α , TNF α ）-磷脂酰肌醇-3-羟激酶（phosphatidylinositol 3-hydroxy kinase, PI3K）-雷帕霉素靶蛋白（mammalian target rapamycin, mTOR）、PKA、PKC三个通路抑制AQP3基因的转录（Asai *et al.*, 2006）。而Nader和Kino等（Kino & Chrousos, 2011; Nader *et al.*, 2009）发现，Clock基因是一个可以调节皮肤24 h节律的基因，时钟基因CLOCK和BMAL1直接结合human AQP3（hAQP3）启动器的响应元件（D-site/E-box）来激活其转录，同时，它可以通过抑制激素受体的转录活性，从而维持激素正常功能，进而调控AQP3的表达（Matsunaga *et al.*, 2014）。故推测，Clock基因通过调节激素受体的活性调节激素达到正常水平，进而通过磷酸化途径影响AQP3基因的转录，从而调控AQP3的表达。

除此之外，血管活性肠肽可增加AQP3的表达；肿瘤坏死因子TNF α 可以抑制AQP3的表达（Cao *et al.*, 2006; Horie *et al.*, 2009; Murai *et al.*, 2008）。

2. Effects of physical and chemical properties of internal and external environment

由于细胞与内环境之间、内环境与外界环境之间不断地进行着物质交换，因此内外环境的不断变化，必然会影响细胞的代谢活动。通过调研发现，AQP3的表达亦受到内外环境理化性质的影响。研究显示，在含有山梨醇、氯化钠、甘露醇及糖类等的渗透压环境下，AQP3 mRNA的表达是增加的，所以可知，AQP3的表达受渗透压环境的影响

（Garcia *et al.*, 2011）。而且，水通道蛋白的表达还受氧浓度的影响（Fujita *et al.*, 2003）。在低氧条件下，AQP3的mRNA及蛋白的表达均出现明显的降低。另外，在pH=6.0的条件下，AQP3表现为甘油通道，而当pH<5.6时，细胞完全失去对水和甘油分子的通透性（Zheng & Bollinger Bollag, 2003），AQP3对水的通透能力是与pH有关的（Németh-Cahalan *et al.*, 2004）。AQP3的通透性还受Cu²⁺和Ni²⁺的抑制作用，分子中的细胞外氨基酸残基Trp128、Ser152和His241与Cu²⁺相关，特别是Ser152还参与酸碱度调控AQP3渗透性的过程（Ikarashi *et al.*, 2012）。所以，AQP3的表达与细胞赖以生存的内环境有密切的联系。

Effects of AQP3 on human skin

1. Relationship between AQP3 and skin moisture

1) The function of transferring water

研究表明（Itoh *et al.*, 2003），AQP3可将体内循环中的水分带入表皮，在表皮细胞基底层至角质层间提供一种短的水回路，从而保证表皮持续的水含量，对于表皮的屏障作用、水的保存有很大的意义。目前已经有研究证实（Bonté, 2011; Zhang & Li, 2013），通过调节AQP3的表达，可以改变干燥性皮肤病的皮肤含水量，减少水分丢失。

2) The function of transferring glycerol

AQP3不仅可以运送水，同时还能从循环中将内源性甘油、皮脂腺中的甘油三酯带入表皮，进而参与表皮细胞甘油代谢，表皮角质形成细胞合成的甘油三酯和皮脂腺分泌的甘油三酯都是角质层中甘油的来源，而角质层甘油的含量是影响皮肤含水量的重要因素之一，对于保持皮肤的含水量也十分重要（Schrader *et al.*, 2012），故AQP3转运甘油成为皮肤保湿的另一途径。

3) Synergistic effects of AQP3 and filaggrin

中间丝相关蛋白是参与皮肤表皮角质包膜形成的重要蛋白，形成表皮角质层独特的屏障结构，从而维持皮肤正常含水量，防止皮肤水分散失。研究者发现（Hara-Chikuma *et al.*, 2015），当用tape stripping破坏皮肤屏障，或者用醚（ether）/丙酮（acetone）去除脂质造成皮肤屏障功能受损时，AQP3表达会立即增高，并且在屏障破坏后24 h增高最明显。而在屏障破坏后3 h，中间丝相关蛋白的表达出现短暂而明显的下降，并在24 h恢复正常。中间丝相关蛋白水解后可提供天然保湿因子（natural moisturizing factor, NMF），主要是氨基酸，L-谷氨酰胺、L-谷氨酸衍生物、吡

咯烷酮羧酸。造成此显现的原因是，当皮肤屏障遭到破坏后，导致渗透压应激，作为缺水的立即反应，中间丝相关蛋白降解产生新的NMF成分，而AQP3则作为迟发的二次反应接替中间丝相关蛋白的作用。因此，AQP3与中间丝相关蛋白的这种协同作用为细胞提供充足的水环境，从而重建皮肤屏障达到保湿功效。

4) Relationship between AQP3 and tight junction protein
紧密连接相关蛋白 (tight junction, TJ) 通常位于上皮顶端两相邻细胞间，在皮肤中的表达对皮肤屏障功能至关重要。张文杰 (Zhang *et al.*, 2012) 等，利用靶向AQP3的shRNA技术，通过 reverse transcription-polymerase chain reaction (RT-PCR) 和Western blot法及免疫细胞化学法检测和观察了干扰AQP3的表达后，其对TJ主要构成蛋白Occludin和Claudin-1的影响并探讨了其可能机制。结果显示，干扰AQP3的表达后，Occludin和Claudin-1的表达明显降低，且TJ结构遭到破坏。推测其可能的机制是，PKC通路在TJ中发挥着重要的作用，Suzuki *et al.* (2009) 发现PKC可使Occludin中C端结构域苏氨酸磷酸化，调节TJ功能；还有研究发现PKC可磷酸化人Claudin-4的第194个丝氨酸，进而影响TJ功能 (Aono & Hirai, 2008)。而AQP3分子中含有蛋白激酶磷酸化位点，因此AQP3也可能是通过PKC通路影响TJ功能，从而影响皮肤屏障功能，进而影响皮肤的保湿，但其具体机制仍需进一步研究。

2. Relationship between AQP3 and skin aging

研究显示 (Tang, 2008)，在不同年龄段，AQP3 mRNA及蛋白在皮肤中的表达是有差异的。Hara-Chikuma *et al.* (2015) 取55例健康人腹部皮肤，按年龄分为青年组 (<20岁)、中年组 (30-45岁) 和老年组 (>55岁)，反转录聚合酶链反应及蛋白质印迹法检测发现，老年组皮肤中AQP3表达量显著低于中年组，中年组皮肤中AQP3显著低于青年组。AQP3 mRNA及蛋白随年龄的增加而逐渐减少。

另外，在一项对AQP3缺乏的小鼠的生物学研究中 (Qin *et al.*, 2011)，当AQP3含量降低时，甘油的新陈代谢和生物合成受损，一方面导致三磷酸腺苷 (adenosine triphosphate, ATP) 含量降低，接着丝裂原激活的蛋白激酶 (mitogen-activated protein kinase, MAPK) 信号也会因此被破坏，从而降低了细胞的增殖能力，促进细胞凋亡；另外，甘油含量的降低也会导致脂类合成受损，从而也会影响细胞增殖。细胞增殖能力降低又会导致AQP3合成减少，如此形成一个循环，促进细胞的死亡。相反，当AQP3含量升高，可以为细胞增殖提供ATP，增强细胞活力，延缓皮肤衰老。

Conclusion

AQP3是皮肤中表达最明显的水通道蛋白亚型。AQP3不仅可以转运水，也可以转运甘油、尿素等小分子物质，与此同时，AQP3也可以影响与皮肤屏障相关的蛋白，如协同中间丝相关蛋白、调节紧密连接相关蛋白的表达，此功能对AQP3维持皮肤表皮的水合作用，修复皮肤屏障具有重要意义。除此之外，AQP3可以为细胞增殖提供ATP，增强细胞活力，降低细胞凋亡，延缓衰老。

另外，本文对AQP3的调控机制进行了深入的总结，时钟基因、磷酸化作用等均可以通过相应通路来调控AQP3的表达。

通过对AQP3的调控机制及对人皮肤的作用的总结，阐明了调节AQP3的表达可以有效的调节皮肤的状态，AQP3作为化妆品领域功效研究新靶点，具有良好的前景。

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中文摘要

水通道蛋白AQP3调控机制及对皮肤作用研究进展

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水通道蛋白3 (AQP3) 在特异性皮炎、白癜风、银屑病等疾病中发挥着重要的作用, 旨在通过AQP3对皮肤疾病的影响, 探索其调控机制及对皮肤的作用。本文总结了AQP3在皮肤中的分布, 及其调控机制, 指出了其对皮肤的影响以及AQP3作为化妆品领域研究新靶点的发展方向。

关键词: 水通道蛋白, 皮肤, 调控, 保湿, 延缓衰老

국문초록

수분통로단백질 AQP3의 조절 메커니즘과 피부작용에 대한 연구

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수분통로단백질인 aquaporin 3 (AQP3)은 백반증, 건선 등 다양한 피부 관련 질병의 발현에 중요한 역할을 한다. 본 연구에서는 AQP3가 피부질환에 미치는 영향을 확인함으로써, AQP3의 피부 속 조절 메커니즘 및 피부작용에 대해 연구하고자 한다. 이를 위해 피부에서 AQP3의 전달 및 기능을 요약한 후 향후 화장품 영역에서 AQP3의 새로운 발전 방향을 밝히고자 한다.

핵심어: 수분통로단백질, 피부, 조절, 보습, 노화지연