Hedgehog与胃腺体分化及Correa级联学说关系的研究进展

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摘要 Hedgehog(Hh)信号通路在胚胎发育、胃腺体分化、解痉多肽表达化生、肠型胃癌进程中发挥关键作用。按胃癌病变的Correa级联学说, 胃癌的发展从慢性萎缩性胃炎、肠化、不典型增生, 最后发展成为肠型腺癌是一个多步的、序贯的过程。该文就Hh信号通路的成员及其通路信号转导, 促进胃腺体分化的分子生物学机制及在Correa级联学说中作用的研究进展作一综述。

关键词 hedgehog; 胃腺体分化; Correa级联学说

Progress in Studies of Relationship of Hedgehog and the Gastric Gland Differentiation, Correa's Cascade

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Abstract Hedgehog (Hh) signaling pathway plays a crucial role in a variety of processes, such as embryonic development, gastric gland differentiation, spasmolytic polypeptide-expressing metaplasia (SPEM), intestinal-type gastric cancer. According to Correa's cascade of gastric carcinogenesis, gastric cancer was believed to develop from a multistep and sequential process from chronic atrophic gastritis (CAG), intestinal metaplasia (IM), dysplasia (DYS) and subsequently to intestinal-type adenocarcinoma. Here, we present an overview of the components and the signal transduction of Hh pathway, the mechanism of Hh signaling pathway in the gastric gland differentiation and the role of Hh pathway in the process of Correa's cascade of gastric carcinogenesis.

Keywords hedgehog; gastric gland differentiation; Correa's cascade

1980年, Nüsslein-Volhard等[1]首先发现*Hedgehog*(*Hh*)基因。根据Correa级联学说[2], 肠型胃癌的发生模式: 一般遵循正常胃黏膜→慢性浅表性胃炎→慢性萎缩性胃炎→肠上皮化生→不典型增生→胃癌的顺序演变。伴随对Hh信号通路研究的逐步深入, 已证明可促进胃腺体分化及异常激活在肠型胃癌发生、发展过成中起关键作用。本文就Hh信号通路特点、促胃腺体分化的分子机制及与胃癌前病变和肠型胃癌发生、发展关系等方面的研究进行综述。

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1 Hh信号通路的构成和信号转导

1.1 Hh信号通路的主要成员

Hh家族在维持胃黏膜正常结构、形态、功能中发挥重要的作用, 在表面黏液细胞、壁细胞、主细胞、颈黏液细胞、胃干细胞的分化、更新、增殖中不可或缺。在果蝇中, 只有一种分泌型Hh配体蛋白激活通路, 转录因子为cubitus interruptus(Ci)并存在两种形式: 全长的转录激活蛋白(Ci155)和N-末端片段截短的转录阻遏蛋白(Ci75), 激酶Fused(Fu)存在于果蝇而不存在于哺乳动物中, 非典

型驱动蛋白为Costal2(Cos2)。在哺乳动物中它包括sonic hedgehog(Shh)、Indian hedgehog(Ihh)和desert hedgehog(Dhh)三个蛋白配体, 人类Hh信号通路主要由相应的分泌型蛋白配体、跨膜蛋白受体1(patched1, Ptchl)和Ptch2、另一跨膜蛋白共受体smoothened(Smo)、glioblastoma(Gli)的调节因子suppressor of fused(SuFu)、驱动蛋白7(kinesin 7, Kif7)以及下游转录因子Gli蛋白家族(Gli1、Gli2、Gli3)等组成[3]。

1.2 Hh信号转导

Hh前体蛋白在内质网中经自身催化水解, 剪切C-端后, 以共价形式连接胆固醇形成N-端肽, 然后通过酰基转移酶在N-端棕榈酰化后形成具有信号转导功能的成熟肽[4-5]。没有Hh配基存在时, 定位在初级纤毛底部的Ptch与Smo形成复合体, Smo的活性被抑制。接受Hh信号后, Hh结合到Ptch, Ptch离开初级纤毛底部, Hh配基和Ptch随后被酶解, Ptch对Smo的抑制作用被解除。活化的Smo则从囊泡转移到初级纤毛上并在初级纤毛上积累[6]。激活的Smo一方面促进Kif7转运到初级纤毛顶端, 另一方面与初级纤毛顶部的Gli-Kif7-SuFu-Fu复合物作用, 促使Gli的激活[7], 进而调控靶基因*Wnt*、*N-myc*、*Ptch1*、*Gli1*等的转录。当Smo被Ptch抑制时, 酪蛋白激酶CKIα、糖原合酶3β(glycogen synthase kinase 3β, GSK3β)和蛋白激酶A(protein kinase A, PKA)等蛋白激酶磷酸化Gli, 随后被β-TrCP(beta-transducin repeat-containing proteins)泛素连接酶识别[8]并酶解加工形成截短的抑制剂形式, 发挥*Hh*靶基因转录抑制作用。

2 胃腺体细胞分布特点及分化机制

2.1 胃腺体细胞分布特点

胃底腺胃小凹由表面上皮组成, 内含多种黏液分泌细胞, 腺体向下深入黏膜, 可分为峡部、颈部和基底部。峡部有颈黏液细胞、干细胞、神经内分泌细胞。颈部有黏液颈细胞(mucous neck cell, MNC)、壁细胞和神经内分泌细胞。基底部主要有主细胞, 偶见壁细胞, 还有一些神经内分泌细胞。幽门腺胃小凹更深, 胃小凹由颈黏液细胞组成。胃小凹下的幽门腺主要为黏液细胞, 偶见胃酶细胞, 罕有壁细胞。内分泌细胞如分泌胃泌素的G细胞、分泌生长抑素的D细胞、分泌组胺的肠嗜铬样细胞等被发现分散在胃腺体中[9-10]。贲门黏膜与幽门黏膜相似。

2.2 胃腺体主要细胞分化机制

胃腺中峡部干细胞主要依靠配体Wnt3A来维持细胞的“干性”[11]。Yang等[12]研究表明, 发育中鼠的胃底腺干细胞最初分散在上皮各层, 随后集中在小凹深层, 随着胃底腺的成熟, 干细胞离开腺体基底部向胃腔方向移居。表面黏液细胞由前小凹上皮细胞转化而来, 表达三叶因子1(trefoil factor 1, TFF1)和叉头框蛋白Q1(forkhead box Q1, FoxQ1)[13], 受Ihh和表皮生长因子(epidermal growth factor, EGF)的调控[14-16]。黏液颈细胞从前颈细胞中转化而来, 能分泌三叶因子2(TFF2)/解痉多肽(spasmolytic polypeptide, SP)、胃蛋白酶原A(pepsinogen A)和pepsinogen C。主细胞产生及分化受视黄酸(retinoic acid, RA)及碱性螺旋–环–螺旋转录因子Mist1调控[16-19], 可由黏液颈细胞诱导分化而来。胃壁细胞由胃前壁细胞进一步分化而来, Shh、骨形态发生蛋白4(bone morphogenetic protein 4, BMP4)、胃泌素(gastrin)调控壁细胞分化[16,20-21], 壁细胞可表达ADP-核糖基化因子1(ADP-ribosy-lation factor 1, ARF1)、钙调蛋白2等蛋白[13,16]。

3 Hh促进胃黏膜分化的分子生物学机制

2014年, McCracken等[22]在人类多能干细胞中添加激活素A(activin A)培育出限定性内胚层。再在内胚层中添加成纤维细胞生长因子4(fibroblast growth factor4, FGF4)、Wnt3A蛋白、RA、头蛋白(noggin)、EGF等在实验室中成功培育出微型胃, 这标志着对胃的胚胎发育机制的研究有了巨大的进展。

3.1 肠分化机制

Activin A可诱导气管、肺、肝等脏器标识基因及八聚体结合转录因子-3/4(octamer binding transcription factor-3/4, Oct-3/4)表达, 驱动人类多能干细胞生成限定性内胚层。在胚胎发育期间内细胞团中, Oct-3/4与性别决定区Y框蛋白2(SRY-related high-mobility group box 2, Sox2)诱导FGF4表达, FGF4可进一步诱导BMP4的表达。Wnt3a、FGF4和BMP4协同诱导表达尾型同源框转录因子2(caudal type homeobox 2, Cdx2)的后肠形成[23-24]。Oct-3/4只在早期胚胎形成过程中表达, 在原肠胚形成过程中下调, 这可能是原肠形成过程中伴随Cdx2表达, Cdx2抑制了Oct-3/4表达的原因。虽然β-catenin可提高Oct-3/4活性[25-26], 但Oct-3/4却可促使β-catenin降解[27], 对

β-catenin有负调节的作用, 故Oct-3/4蛋白水平减少, β-catenin蛋白水平增加[27], 导致早期胚胎干细胞分化期间经典Wnt的信号通路上调促进肠的分化。

3.2 胃分化机制

在人类多能干细胞中添加activin A、Wnt3a及FGF4培育出肠组织[23]实验基础上, McCracken等[22]进一步添加RA、Noggin、EGF等在实验室中成功的培育出微型胃。该实验中添加RA为促进前肠尾部发育所必需[28]。Hu等[29]研究发现, RA限制Wnt/β-catenin信号通路, 可通过激活N-myc下游调控基因1a(N-myc downstream regulated gene 1a, *Ndrg1a*)限制经典的Wnt信号通路促进胃的分化[30]。添加Noggin的目的是限制BMP4信号通路, 导致后肠标识Cdx2的表达受到抑制[31-32], 激活前肠标识Sox2的表达[22,33-34]。最终在Shh、Sox2、RA、FGF10等蛋白的作用下, 在实验室中把人类多能干细胞培育成“迷你胃”。

在实验小鼠以及培养的小鼠胃和肠道组织实验中, 转录因子Barx1驱动胃黏膜分化。Kim等[35]报道, Wnt信号通路调节胃肠黏膜发育的早期阶段, 引起上皮细胞增殖, 随后位于肠黏膜下方间充质细胞表达Barxl。Barxl介导间充质细胞表达分泌型Frz相关蛋白(secreted frizzled-related proteins, Sfrps), Sfrps拮抗下调经典Wnt信号通路, 促进胃上皮的发育。在小鼠胚胎发育中, 前肠尾部经历了演变, 再分化为表达$Sox2^+$/胰十二指肠同源盒基因1(pancreatic and duodenal homeobox 1, *Pdx1*)的胃底、表达$Sox2^+$/$Pdx1^+$的胃窦、表达Pdx1/胰腺特定转录因子1a(pancreas specific transcription factor 1a, Ptf1a)的胰腺及表达Pdx1/$Cdx2^+$的十二指肠[22]。动物实验表明, Hh信号可以抑制Pdx1表达[36], 胃底不表达Pdx1, 可能与胃体、底的壁细胞分泌Shh抑制了Pdx1有关。

3.3 Shh促进胃腺体分化机制

在Roberts等[37]研究中, Shh在除了胰腺以外的所有内胚层中表达。Hh为作用于内胚层和间质细胞之间的内胚层的信号分子, 激活Hh信号通路可以促进胃干细胞分化、更新。Shh和Ihh在胃肠及腺体中的表达分布特点为: 在胃肠中, Shh优势表达在胃中, 在肠道中尤其小肠中表达很低。Ihh在幽门及十二指肠中高度表达。在胃中, Shh优势表达在胃体、胃底; Ihh优势表达在胃窦、幽门。在胃腺体中, Shh优势表达在胃腺体腔内, Ihh优势表达在小凹上皮细胞中。Shh在胃腺体颈区及峡部的干细胞区中表达强度最高, 逐渐向腺体的基底部递减, 呈梯度分布趋势。Ptch1在胃腺体峡部不表达[14]。

Shh为胃腺体分化的核心蛋白[38], Shh和Wnt在形态发生中相互拮抗[39]。Shh可以通过以下途径抑制Wnt信号通路, 促进胃腺体分化, 主要包括: (1)Shh可被胃间质中FGF10激活, 进而促进BMP4表达[38]。BMP4通过激活Barxl表达[38,40]及磷酸 化Pten(phosphatase and tensin homolog deleted on chromosome ten)阻断Akt信号通路, 来抑制经典的Wnt信号通路的活性水平。(2)Hh可通过激活Sfrp-1的表达抑制Wnt信号通路[41]。(3)Foxl1是Wnt信号通路的负性调节因子, $Foxl1^{-/-}$小鼠肠隐窝细胞核β-cat增多, 肠隐窝面积增大[42-43]。Shh可促进胃肠道间叶细胞表达Foxl1来抑制Wnt信号通道。Foxl1还通过上皮–间叶交互作用而促进壁细胞分化[44-45], 此外, Shh还可以通过以下非抑制Wnt信号通路方式促进胃腺体分化, 主要包括: (1)Shh可激活胃腺体标识基因Sox2表达[46], 决定胃腺体分化。(2)Shh的表达与胃底腺的分化相关[47], 它可能通过调节叉头框蛋白A2(forkhead box A2, FoxA2), 胰岛素基因增强子结合蛋白1(insulin gene enhancer protein 1, Isl1)及间叶细胞中BMP4的表达驱动胃底腺体分化[48]。(3)Shh可激活Foxa1(HNF-3α)促使表面黏液细胞表达TFF1。在人和两栖动物的三叶因子启动子中具有一个与HNF-3结合位点惊人相似性的接近TATAA盒的序列, 可能对三叶因子基因转录产生影响[49]。(4)Shh可以激活Foxq1表达, Foxq1上调可以导致Cdx2表达缺失。Foxq1能影响表面黏液细胞的分化。Muc5ac为表面黏液细胞分泌, 缺乏Foxq1鼠表面黏液细胞的分化障碍可导致Muc5ac完全缺乏[50], 激活Foxq1可促使Muc5ac表达。(5)Shh与胃泌素、BMP4诱导壁细胞分化。BMP4能增加壁细胞的H^+,K^+-ATP酶表达[51]。在犬胃壁细胞中, Shh信号通路在Akt的调节下能介导EGF在壁细胞中发挥活性作用, 促进壁细胞中的H^+,K^+-ATP酶a亚单元基因表达, 增强由胃泌素刺激的胃酸分泌, 促进壁细胞的成熟和分化[20]。与Shh不同, Ihh在表面黏液细胞中表达, 促进及维持表面黏液细胞分化、生存[14,52]。(6)Shh可以激活KLF4(kruppel-like factor 4)的表达[46]。研究发现, KLF4表达缺失的小鼠胃上皮壁细胞和成熟主细胞的数量减少一半以上, 而颈黏液细胞的数量上升4倍[53]。总之, Wnt/Cdx2信号通路和Barx1/

Sfrp/Sox2信号通路之间的相互拮抗, 优势表达信号通路决定细胞向胃或肠的方向分化。

4 Hh与胃黏膜萎缩、化生及不典型增生的分子生物学机制

4.1 Shh与胃黏膜萎缩的发生机制

Correa级联学说肠型胃癌序贯发生途径的总体正确性已经被小鼠模型证实[54]。萎缩性胃炎为固有腺成分减少, 被基质纤维化和/或肠上皮化生或假幽门腺化生所替代, 壁细胞也随之减少。壁细胞不仅具有泌酸、产生内因子等功能, 而且在胃黏膜细胞的增生和分化中起关键作用。炎症因子特别是白细胞介素-lβ(interleukin-lβ, IL-lβ)具有强大的抑制胃酸分泌的作用, IL-lβ强烈的抑酸作用可导致胃黏膜萎缩[55], 机制之一是通过抑制Shh来实现的[56]。IL-1β强烈的抑酸作用升高了胃内pH值, 限制了Shh的表达, Shh的丢失先于壁细胞的减少。在成人的胃中, 壁细胞表达Shh蛋白和mRNA[47-48], Shh也为壁细胞分化所必需。IL-1β下调*Shh*基因表达最终导致壁细胞减少。因壁细胞的协同作用为主细胞分化所必需[57], 故壁细胞的减少会影响主细胞分化, 主细胞也随之减少。另外, 壁细胞表达furin, furin为加工前体蛋白的费林蛋白酶, furin可介导转化生长因子-α(transforming growth factor-α, TGF-α)和EGFR作用于黏膜上皮细胞的更新[58]。壁细胞减少影响胃黏膜上皮细胞更新, 最终导致胃黏膜萎缩, 萎缩性胃炎发生。

4.2 Shh与肠上皮化生的机制

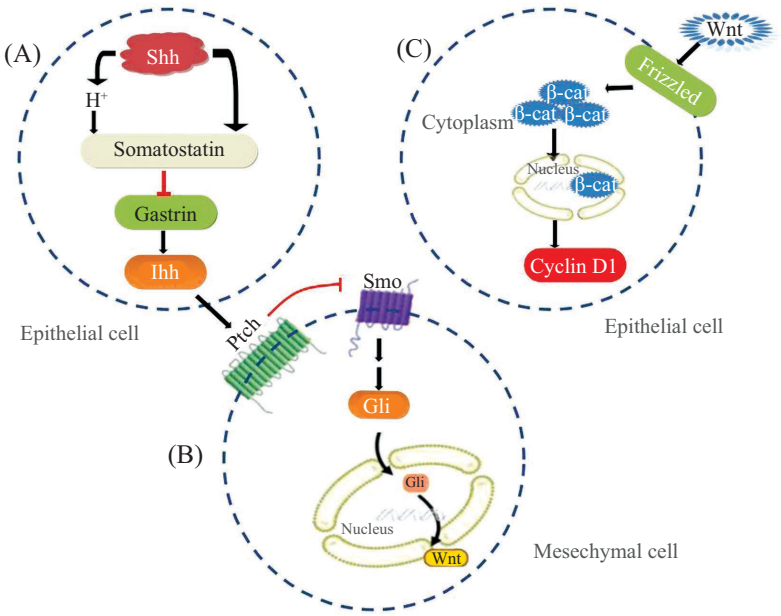
目前, 研究认为, 化生代表着发生化生的前体上皮细胞在遗传学/后天程序中最早出现的永久性改变[59]。肠上皮化生(intestinal metaplasia, IM)是指胃黏膜上皮细胞被类似小肠或大肠黏膜的上皮细胞替代, 主要由吸收细胞组成, 其间夹杂有杯状细胞, 底部可见潘氏细胞。正常情况下, Cdx2特异性表达于肠黏膜, 正常胃黏膜不表达, Cdx2可异位表达于肠化生上皮、胃腺癌和人胃腺癌细胞株。研究发现, *Shh*基因在萎缩性胃炎及肠上皮化生中表达下调[60], *Shh*基因敲除的小鼠, 胃黏膜上皮被能分泌碱性磷酸酶的肠上皮所替代[61]。其机制可能为, 下调Shh引起胃黏膜标识基因*Sox2*表达受到抑制, 这也导致Wnt信号通路活性增强。增强的Wnt信号促进了Notch信号通路的活性, 激活了靶基因*Math1*及*Hes1*表达, 进一步与FGF4、BMP4协同作用促进吸收细胞、潘氏细胞、杯状细胞产生, 启动了肠黏膜标志基因Cdx2的表达, 胃黏膜的肠转分化。Cdx2在低级别上皮内瘤变、高级别上皮内瘤变和肠型胃癌中分别有73.3%、85.5%、91.1%表达, 在肠上皮化生的胃黏膜标本89.7%为阳性, 而在正常胃黏膜不表达, 提示Cdx2的表达是胃黏膜癌变过程中的早期事件[62]。

4.3 假幽门腺化生的特征

胃体、胃底出现的假幽门腺化生类似于胃窦或幽门腺的表现, 其特点是表达TFF2/SP以及主细胞标志物——胃蛋白酶原I。解痉多肽表达化生(spasmolytic polypeptide-expressing metaplasia, SPEM)被认为是一种胃体黏膜损害, 它比IM更广泛与胃癌相联系[63-64], 通常IM和SPEM一起出现[65-66]。SPEM病理特点为表达TFF2, 黏液细胞增多而成熟壁细胞、主细胞丢失。TFF2常由黏液颈细胞及胃窦腺细胞表达[63], 也可由主细胞转化来的细胞或者激活的隐窝基底部祖细胞来表达[67-68]。使用敲出Mist1-cre小鼠研究发现, 主细胞转化为表达TFF2的细胞[69]。当BMP信号通路的拮抗剂Noggin在壁细胞中过表达, 壁细胞减少, 表达TFF2黏液细胞增多[70]。胃体、胃底中Shh减少, 会导致相应的胃腺体中壁细胞减少, 假幽门腺化生发生。

4.4 Hh与不典型增生的发生机制

胃黏膜不典型增生, 又称上皮异型增生, 是胃癌的重要癌前病变。Dimmler等[71]在体外实验发现, 低pH值环境促进*Shh*基因在胃癌细胞系23132中的表达; 而在pH值升高的情况下, *Shh*基因的表达明显下调。药物限制小鼠中Hh信号通路可导致表皮细胞增殖增加60%~70%[48]。在小鼠胃黏膜细胞中, Ihh在小凹上皮细胞中优势表达, 它诱导了小凹上皮细胞(隐窝细胞)的分化; Shh在腺体里表达, 与Ihh形成动态平衡[14]。在表面隐窝上皮中, 增加*Ihh*基因表达, 锌指转录因子Snail增加近40倍, E-cadherin减少显著, 因为Snail抑制了上皮细胞钙黏附蛋白(E-cadherin, E-cad)的表达[72-74], 进而导致上皮–间质转化(epithelial-mesenchymal transition, EMT)。然而, Shh却通过诱导上皮细胞钙黏着蛋白(E-cadherin)表达促进上皮细胞的分化[75]。在成年鼠胃壁细胞中Shh丢失可导致胃酸过少, 生长抑素也随之减少, 继之胃泌素增加, 在表面隐窝上皮中, 高胃泌素血症诱导表面黏液细胞内Ihh表达, Ihh在细胞间质中与Ptch受体结合, 诱



A: Shh调控Ihh的机制; B: Ihh信号诱导Wnt蛋白表达; C: 经典Wnt通路被激活。

A: the mechanism of regulating Ihh by Shh; B: Ihh induces expression of Wnt; C: activation of the canonical Wnt pathway.

图1 Shh丢失导致表面黏液细胞增殖的可能机制(根据参考文献[76]修改)

Fig.1 Proposed mechanism for the development of hyperproliferation in the surface mucous cells with loss of Shh (modified from reference [76])

导Gli1迁移至细胞核, 激活靶基因诱导Snail和Wnt蛋白表达, 在上皮细胞中通过经典Wnt信号通道激活细胞周期蛋白D1, 导致细胞增殖[76](图1), 这可能是慢性炎症中胃黏膜不典型增生的原因之一。

5 慢性炎症中Shh与胃癌发生的机制

慢性炎症与癌变之间存在关联, IL-1β、IL-6、环氧合酶2、肿瘤坏死因子等可能是慢性炎症演化为致癌环境中最核心的炎症介质。其中, 骨髓源细胞(bone marrow-derived cells, BMDCs)是IL-1β主要来源[77], 即使没有幽门螺杆菌感染, IL-1β过表达的转基因小鼠仍可发生癌症, 这证明了慢性炎症在癌变中发挥重要作用。

5.1 组织中Shh、Gli的分布表达特点

Shh具有促进胃黏膜分化与胃癌发生的双重作用, 二者信号转导通路是完全不同的。后者是在慢性炎症微环境作用下主要通过经典Shh-Gli1信号通路途径或非经典的Hh信号通路来实现的。这与Wnt信号通路一方面促进肠黏膜分化, 另一方可促进各种癌的发生相似。Shh与其靶基因*Gli1*的表达往往具有一致性, 但是Shh的高表达并不总是伴随Gli1的高表达。在胎鼠原位杂交实验中, *Gli*基因的表达在整个胚胎发育过程中都被局限在间叶组织中[78], 在不同胚胎组织中的表达形式似乎都遵循相似的规则。在表达Shh的细胞中, *Gli*基因从不被激活。*Gli1*基因的表达通常在表达Shh细胞的附近被发现, 而*Gli3*基因却恰好不在这些细胞中表达。

5.2 Gli1与Gli2分别在胃体、胃窦癌前病变中的作用

Shh的靶基因*Gli1*在不论是否感染的正常胃黏膜中都是低表达的, 然而却在化生阶段又重新激活而高表达[79]。慢性胃炎胃体幽门腺化生或者SPEM, Gli1表达是必需的[80]。在研究Hh在HP(helicobacter pylori)感染性胃炎中的作用时, El-Zaatari等[80]感染HPC57BL/6野生型和Gli1$^{-/-}$鼠2个月、6个月, 仅仅野生型鼠6个月时间发展成为SPEM, Gli1$^{-/-}$鼠不能发展成为化生性改变。*Gli1*敲除可以预防胃黏膜化生。研究发现, 与胃体中依赖炎症调控的Gli1比较, 使用缺乏胃泌素的lacZ报告鼠的胃窦黏膜增生的原因是Gli2而不是Gli1[81-82], 胃泌素的丢失容易发生癌变的是胃窦而不是胃体[83]。故在胃窦, Hh相关转录因子诱导癌前病变是Gli2而不是Gli1。

5.3 慢性炎症中Shh与胃癌发生的机制

慢性炎症中, Shh配体作为化学诱导物募集炎症细胞迁移至胃部[84], 一方面炎症因子抑制壁细胞分泌胃酸导致壁细胞与主细胞减少, 另一方面

炎症因子可导致间充质细胞增多产生大量的增殖基质因子, 例如, Wnts、TGF-β能有效地诱导黏液细胞肠化、增生及癌变。Song等[85]发现, Shh信号通路的靶基因Ptchl、Gill不管是mRNA水平还是蛋白质水平, 在胃癌干细胞中均高表达。前炎症因子Th1也可刺激间质细胞表达Shh。来自骨髓间充质干细胞被招募到胃中, 也表达Shh[86]。这样的病理环境容易引起Shh-Gli1信号通路的异常激活, 导致胃癌的发生。HP(Helicobacter pylori)感染导致的慢性胃炎中, $CD11b^{+}CD11c^{-}Slfn4^{-}$(Schlafen4^{-})骨髓源细胞也可从骨髓迁移至胃。骨髓源细胞表达Gli1。Gli1可促使$CD11b^{+}CD11c^{-}Slfn4^{-}$细胞转化为$CD11b^{+}CD11c^{+}Slfn4^{+}$细胞。表面有$CD45^{+}$主要组织相容性复合体$II^{+}$(major histo compatibility complexII^{+}, $MHCII^{+}$)$CD11b^{+}CD11c^{+}Slfn4^{+}$表达的骨髓源细胞亚群, 类似骨髓源抑制细胞(myeloid-derived supp ressor cells, MDSCs)[80]。胃黏膜化生与$CD45^{+}MHCII^{+}CD11b^{+}CD11c^{+}$骨髓源细胞出现一致。Gli1可促使$CD45^{+}MHCII^{+}CD11b^{+}CD11c^{+}Slfn4^{+}$细胞表达IL-1β和TNF-α。而IL-1β、TNF-α过表达与胃癌的发生密切相关。Houghton等[87]研究发现, BMSCs可形成胃癌, 给胃癌研究带来极大启发。但Marx等[88]认为, Houghton等的研究并没有提供充足的证据来证明骨髓来源的干细胞确实是分化为上皮细胞还是和上皮细胞融合, 因为骨髓来源的干细胞易与其他类型细胞发生融合。Cao等[89]从胃癌组织里分离出了间充质干细胞, 并对间充质干细胞的特性进行了鉴定, 结果与骨髓间充质干细胞相似, 但是与胃癌细胞不同。以上研究表明, BMDCs出现在胃部慢性炎症中导致经典Shh-Glil信号通路或者非经典的Hh信号通路激活与胃癌的发生密切相关。

6 小结

形态发生素Hh蛋白家族通过形成浓度梯度来调控下游基因的表达, 主要功能是在胚层中诱导特殊细胞表型和组织间创造一个分界线, 与胃腺体分化、胃黏膜萎缩、肠化、假幽门腺化及肠型胃癌的发生密切相关, 尤其Shh信号, 可以对胃的多种生理及病理现象给出合理解释, 本文就Hh与胃黏膜分化及与Correa级联学说关系的研究进展作一综述, 以期给胃癌的机理研究及防治提供新的思路。

参考文献 (References)

1 Nusslein-Volhard C,Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. Nature 1980; 287(5785): 795-801.

2 Correa P. Human gastric carcinogenesis: A multistep and multifactorial process–first American cancer society award lecture on cancer epidemiology and prevention. Cancer Res 1992; 52(24): 6735-40.

3 Katoh Y, Katoh M. Hedgehog signaling pathway and gastrointestinal stem cell signaling network. Int J Mol Med 2006 18(6): 1019-23.

4 Ok CY, Singh RR, Vega F. Aberrant activation of the hedgehog signaling pathway in malignant hematological neoplasms. Am J Pathol 2012; 180(1): 2-11.

5 Mann RK, Beachy PA. Novel lipid modifications of secreted protein signals. Annu Rev Biochem 2004; 73: 891-923.

6 Nozawa YI, Lin C, Chuang PT. Hedgehog signaling from the primary cilium to the nucleus: An emerging picture of ciliary localization, trafficking and transduction. Curr Opin Genet. Dev 2013; 23: 429-37.

7 Merchant JL. Hedgehog signaling in gut development, physiology and cancer. J Physiol 2012; 590: 421-32.

8 Jiang J, Struhl G. Regulation of the hedgehog and wingless signaling pathways by the F-box/WD40-repeat protein slimb. Nature1998; 391(6666): 493-6.

9 Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E. The “normal” endocrine cell of the gut: Changing concepts and new evidences. Ann NY Acad Sci 2004; 1014: 1-12.

10 Scoville DH, Sato T, He XC, Li L. Current view: Intestinal stem cells and signaling. Gastroenterology 2008; 134(3): 849-64.

11 Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, *et al*. Lgr5 (+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. Cell Stem Cell 2010; 6(1): 25-36.

12 Yang DH, Tsuyama S, Ge YB, Wakamatsu D, Ohmori J, Murata F. Proliferation and migration kinetics of stem cells in the rat fundic gland. Histol Histopathol 1997; 12(3): 719-27.

13 Khurana S, Mills JC. The gastric mucosa development and differentiation. Prog Mol Biol Transl Sci 2010; 96: 93-115.

14 Fukaya M, Isohata N, Ohta H, Aoyagi K, Ochiya T, Saeki N, *et al*. Hedgehog signal activation in gastric pit cell and in diffuse-type gastric cancer. Gastroenterology 2006; 131(1): 14-29.

15 Nomura S, Settle SH, Leys CM, Means AL, Peek RM Jr, Leach SD, *et al*. Evidence for repatterning of the gastric fundic epithelium associated with Menetrier’s disease and TGF alpha overexpression. Gastroenterology 2005; 128(5): 1292-305.

16 Han ME, oh SO. Gastric stem cells and gastric cancer stem cells. Anat Cell Biol 2013; 46(1): 8-18.

17 Ramsey VG, Doherty JM, Chen CC, Stappenbeck TS, Konieczny SF, Mills JC. The maturation of mucus-secreting gastric epithelial progenitors into digestive-enzyme secreting zymogenic cells requires Mist1. Development 2007; 134(1): 211-22.

18 Tian X, Jin RU, Bredemeyer AJ, Oates EJ, Błazewska KM, McKenna CE, *et al*. RAB26 and RAB3D are direct transcriptional targets of MIST1 that regulate exocrine granule maturation. Mol Cell Biol 2010; 30(5): 1269-84.

19 Karam SM, John R, Alpers DH, Ponery AS. Retinoic acid

stimulates the dynamics of mouse gastric epithelial progenitors. Stem Cells 2005; 23(3): 433-41.

20 Stepan V, Ramamoorthy S, Nitsche H, Zavros Y, Merchant JL, Todisco A. Regulation and function of the sonic hedgehog signal transduction pathway in isolated gastric parietal cells. J Biol Chem 2005; 280(16): 15700-8.

21 Shinohara M, Mao M, Keeley TM, El-Zaatari M, Lee HJ, Eaton KA, *et al*. Bone morphogenetic protein signaling regulates gastric epithelial cell development and proliferation in mice. Gastroenterology 2010; 139(6): 2050-60.

22 McCracken KW, Cata EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, *et al*. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature 2014; 516(7531): 400-4.

23 Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, *et al*. Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. Nature 2011; 470(7332): 105-9.

24 Green MD, Chen A, Nostro MC, d'Souza SL, Schaniel C, Lemischka IR, *et al*. Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells. Nature Biotechnol 2011; 29(3): 267-72.

25 Kelly KF, Ng DY, Jayakumaran G, Wood GA, Koide H, Doble BW. β-catenin enhances Oct-4 activity and reinforces pluripotency through a TCF-independent mechanism. Cell Stem Cell 2011; 8(2): 214-27.

26 Li J, Li J, Chen B. Oct4 was a novel target of Wnt signaling pathway. Mol Cell Biochem 2012; 362(1/2): 233-40.

27 Abu-Remaileh M, Gerson A, Farago M, Nathan G, Alkalay I, Zins Rousso S, *et al*. Oct-3/4 regulates stem cell identity and cell fate decisions by modulating Wnt/β-catenin signalling. EMBO J 2010; 29(19): 3236-48.

28 Bayha E, Jorgensen MC, Serup P, Grapin-Botton A. Retinoic acid signaling organizes endodermal organ specification along the entire antero-posterior axis. PLoS One 2009; 4(6): e5845.

29 Hu X, Gao J, Liao Y, Tang S, Lu F. Retinoic acid alters the proliferation and survival of the epithelium and mesenchyme and suppresses Wnt/β-catenin signaling in developing cleft palate. Cell Death Dis 2013; 4(10): e898.

30 Zhang T, Guo X, Chen Y. Retinoic acid-activated Ndrg1a represses Wnt/β-catenin signaling to allow xenopus pancreas, oesophagus, stomach, and duodenum specification. PLoS One 2013; 8(5): e65058.

31 Barros R, Pereira B, Duluc I, Azevedo M, Mendes N, Camilo V, *et al*. Key elements of the BMP/SMAD pathway co-localize with CDX2 in intestinal metaplasia and regulate CDX2 expression in human gastric cell lines. J Pathol 2008; 215(4): 411-20.

32 Gopal A, Iyer SC, Gopal U, Devaraj N, Halagowder D. Shigella dysenteriae modulates BMP Pathway to induce mucin gene expression *in vivo* and *in vitro*. PLoS One 2014; 9(11): e111408.

33 Green MD, Chen A, Nostro MC, d'Souza SL, Schaniel C, Lemischka IR, *et al*. Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells. Nat Biotechnol 2011; 29(3): 267-72.

34 Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, *et al*. *In vitro* generation of human pluripotent stem cell derived lung organoids. eLife 2015; 4: e05098.

35 Kim BM, Buchner G, Miletich I, Sharpe PT, Shivdasani RA. The stomach mesenchymal transcription factor Barx1 specifies gastric epithelial identity through inhibition of transient Wnt signaling. Dev Cell 2005; 8(4): 611-22.

36 Zhang J, Rosenthal A, de Sauvage FJ, Shivdasani RA. Downregulation of Hedgehog signaling is required for organogenesis of the small intestine in *Xenopus*. Dev Biol 2001; 229(1): 188-202.

37 Roberts DJ, Johnson RL, Burke AC, Nelson CE, Morgan BA, Tabin C. Sonic hedgehog is an endodermal signal inducing Bmp-4 and Hox genes during induction and regionalization of the chick hindgut. Development 1995; 121(10): 3163-74.

38 Zorn AM, Wells JM. Vertebrate endoderm development and organ formation. Annu Rev Cell Dev Bio 2009; 25: 221-51.

39 Aviles EC, Wilson NH, Stoeckli ET. Sonic hedgehog and Wnt: Antagonists in morphogenesis but collaborators in axon guidance. Front Cell Neurosci 2013; 7: 86.

40 Smith TM, Lozanoff S, Iyyanar PP, Nazarali AJ. Molecular signaling along the anterior-posterior axis of early palate development. Front Physiol 2013; 3: 488.

41 Katoh Y, Katoh M. WNT antagonist, SFRP1, is hedgehog signaling target. Int J Mol Med 2006; 17(1): 171-5.

42 Perreault N, Katz JP, Sackett SD, Kaestner KH. Foxl1controls the Wnt/β-catenin pathway by modulating the expression of proteoglycans in the gut. J Biol Chem 2001; 276(46): 43328-33.

43 Takano-Maruyama M, Hase K, Fukamachi H, Kato Y, Koseki H, ohno H. Foxl1 deficient mice exhibit aberrant epithelial cell positioning resulting from dysregulated EphBÆphrinB expression in the small intestine. Am J Physiol Gastrointest Liver Physiol 2006; 291(1): G163-70.

44 Fukamachi H, Fukuda K, Suzuki M, Furumoto T, Ichinose M, Shimizu S, *et al*. Mesenchymal transcription factor Fkh6 is essential for the development and differentiation of parietal cells. Biochem Biophys Res Commun 2001; 280(4): 1069-76.

45 Kaestner KH, Silberg DG, Traber PG, Schütz G. The mesenchymal winged helix transcription factor Fkh6 is required for the control of gastrointestinal proliferation and differentiation. Genes Dev 1997; 11(12): 1583-95.

46 Moon JH, Heo JS, Kim JS, Jun EK, Lee JH, Kim A, *et al*. Reprogramming fibroblasts into induced pluripotent stem cells with Bmi1. Cell Res 2011; 21(9): 1305-15.

47 van den Brink GR, Hardwick JC, Nielsen C, Xu C, ten Kate FJ, Glickman J, *et al*. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. Gut 2002; 51(5): 628-33.

48 van den Brink GR, Hardwick JC, Tytgat GN, Brink MA, Ten Kate FJ, Van Deventer SJ, *et al*. Sonic hedgehog regulates gastric gland morphogenesis in man and mouse. Gastroenterology 2001; 121(2): 317-28.

49 Becks, Sommer P, dos Santos Silva E, Blin N, Gött P. Hepatocyte nuclear factor 3 (winged helix domain) activates trefoil factor geneTFF1 through a binding motif adjacent to the TATAA box. DNA Cell Biol 1999; 18(2): 157-64.

50 Verzi MP, Khan AH, Ito S, Shivdasani RA.Transcription factor foxq1 controls mucin gene expression and granule content in mouse stomach surface mucous cells. Gastroenterology 2008; 135(2): 591-600.

51 Nitsche H, Ramamoorthy S, Sareban M, Pausawasdi N, Todisco A. Functional role of bone morphogenetic protein-4 in isolated canine parietal cells. Am J Physiol Gastrointest Liver Physiol 2007; 293(3): G607-14.

52 van den Brink GR. Hedgehog signaling in development and homeostasis of the gastrointestinal tract. Physiol Rev 2007; 87(4): 1343-75.

53 KatzJP, Perreault N, Goldstein BG, Actman L, McNally SR, Silberg DG, *et al*. Loss of KLF4 in mice Causes altered proliferation and differentiation and precancerous changes in the adult stomach. Gastroenterology 2005; 128(4): 935-45.

54 Cai X, Carlson J, Stoicov C, Li H, Wang TC, Houghton J. Helicobacter felis eradication restores normal architecture and inhibits gastric cancer progression in C57BL/6 mice. Gastroenterology 2005; 128(7): 1937-52.

55 Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, Sugimura H. Interleukin 1beta poly-morphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. Gastroenterology 2002; 123(1): 92-105.

56 Waghray M, Zavros Y, Saqui-Salces M, El-Zaatari M, Alamelumangapuram CB, Todisco A, *et al*. Interleukin-1beta promotes gastric atrophy through suppression of Sonic Hedgehog. Gastroenterology 2010; 138(2): 562-72,572.e1-2.

57 Bredemeyer AJ, Geahlen JH, Weis VG, Huh WJ, Zinselmeyer BH, Srivatsan S, *et al*. The gastric epithelial progenitor cell niche and differentiation of the zymogenic (chief) cell lineage. Dev Biol 2009; 325(1): 211-24.

58 Kamimura H, Konda Y, Yokota H, Takenoshita S, Nagamachi Y, Kuwano H, *et al*. Kex2 family endoprotease furin is expressed specifically in pit-region parietal cells of the rat gastric mucosa. Am J Physiol 1999; 277(1Pt1): G183-90.

59 Takaishi S, Okumura T, Wang TC. Gastric cancer stem cells. J Clin Oncol 2008; 26(17): 2876-82.

60 Shiotani A, Iishi H, Uedo N, Ishiguro S, Tatsuta M, Nakae Y. Evidence that loss of sonic hedgehog is an indicator of Helicobater pylori-induced atrophic gastritis progressing to gastric cancer. Am J Gastroenterol 2005; 100(3): 581-7.

61 Ramalho-Santos M, Melton DA, McMahon AP. Hedgehog signals regulate multipleaspects of gastrointestinal development. Development 2000; 127(12): 2763-72.

62 Kim HS, Lee JS, Freund JN, Min KW, Lee JS, Kim W, *et al*. CDX-2 homeobox gene expression in human gastric carcinoma and precursor lesions. J Gastroenterol Hepatol 2006; 21(2): 438-42.

63 Goldenring JR, Nam KT, Wang TC, Mills JC, Wright NA. Spasmolytic polypeptide-expressing metaplasia and intestinal metaplasia: Time for reevaluation of metaplasias and the origins of gastric cancer. Gastroenterology 2010; 138(7): 2207-10,2210.e1.

64 Lennerz JK, Kim SH, Oates EL, Huh WJ, Doherty JM, Tian X, *et al*. The transcription factor MIST1 is a novel human gastric chief cell marker whose expression is lost in metaplasia, dysplasia, and carcinoma. Am J Pathol 2010; 177(3): 1514-33.

65 Lee HJ, Nam KT, Park HS, Kim MA, Lafleur BJ, Aburatani H, *et al*. Gene expression profiling of metaplastic lineages identifies CDH17 as a prognostic marker in early stage gastric cancer. Gastroenterology 2010; 139(1): 213-25.e3.

66 Nozaki K, Ogawa M, Williams JA, Lafleur BJ, Ng V, Drapkin RI, *et al*. A molecular signature of gastric metaplasia arising in response to acute parietal cell loss. Gastroenterology 2008; 134(2): 511-22.

67 Nam KT, Varro A, Coffey RJ, Goldenring JR. Potentiation of oxyntic atrophy-induced gastric metaplasia in amphiregulin-deficient mice. Gastroenterology 2007; 132(5): 1804-19.

68 Qiao XT, Ziel JW, McKimpson W, Madison BB, Todisco A, Merchant JL, *et al*. Prospective identification of a multilineage progenitor in murine stomach epithelium. Gastroenterology 2007; 133(6): 1989-98.

69 Nam KT, Lee HJ, Sousa JF, Weis VG, O'Neal RL, Finke PE, *et al*. Mature chief cells are cryptic progenitors for metaplasia in the stomach. Gastroenterology 2010; 139(6): 2028-37.e9.

70 Shinohara M, Mao M, Keeley TM, El-Zaatari M, Lee HJ, Eaton KA, *et al*. Bone morphogenetic protein signaling regulates gastric epithelial cell development and proliferation in mice. Gastroenterology 2010; 139(6): 2050-60.e2.

71 Dimmler A, Brabletz T, Hlubek F, Häfner M, Rau T, Kirchner T, *et al*. Transcription of sonic hedgehog, a potential factor for gastric morpnogenesls and gastrlc mucosa maintenance,1s up–regulated in acidic conditions. Lab Invest 2003; 83(12): 1829-37.

72 Medici D, Hay E, Olsen BR. Snail and Slug promote epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3. Mol Biol Cell 2008; 19(11): 4875-87.

73 Tanaka M, Kitajima Y, Edakuni G, Sato S, Miyazaki K. Abnormal expression of E-cadherin and beta-catenin may be a molecular marker of sub-mucosal invasion and lymph node metastasis in early gastric cancer. Br J Surg 2002; 89(2): 236-44.

74 Li X, Deng W, Lobo-Ruppert SM, Ruppert JM. Gli1 acts through Snail and E-cadherin to promote nuclear signaling by beta-catenin. Oncogene 2007; 26(31): 4489-98.

75 Xiao C, Ogle SA, Schumacher MA, Schilling N, Tokhunts RA, Orr-Asman MA, *et al*. Hedgehog signaling regulates E-cadherin expression for the maintenance of the actin cytoskeleton and tight junctions. Am J Physiol Gastrointest Liver Physiol 2010; 299(6): G1252-65.

76 Xiao C, Ogle SA, Schumacher MA, Orr-Asman MA, Miller ML, Lertkowit N, *et al*. Loss of parietal cell expression of Sonic hedgehog induces hypergastrinemia and hyperproliferation of surface mucous cells. Gastroenterology 2010; 138(2): 550-61.e1-8.

77 Franchi L, Amer A, Body-Malapel M, Kanneganti TD, Ozören N, Jagirdar R, *et al*. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. Nat Immunol 2006; 7(6): 576-82.

78 Büscher D, Rüther U. Expression profile of Gli family members and Shh in normal and mutant mouse limb development. Dev Dyn 1998; 211(1): 88-96.

79 El-Zaatari M, Tobias A, Grabowska AM, Kumari R, Scotting PJ, Kaye P, *et al*. De-regulation of the sonic hedgehog pathway in the InsGas mouse model of gastric carcinogenesis. Br J Cancer 2007; 96(12): 1855-61.

80 El-Zaatari M, Kao JY, Tessier A, Bai L, Hayes MM, Fontaine C, *et al*. Gli1 deletion prevents helicobacter-induced gastric metaplasia and expansion of myeloid cell subsets. PLoS One

2013; 8(3): e58935.

81 Saqui-Salces M, Coves-Datson E, Veniaminova NA, Waghray M, Syu LJ, Dlugosz AA, *et al*. Inflammation and Gli2 suppress gastrin gene expression in a murine model of antral hyperplasia. PLoS One 2012; 7(10): e48039.

82 Merchant JL, Saqui-Salces M. Inhibition of Hedgehog Signaling in the gastrointestinal tract: Targeting the cancer microenvironment. Cancer Treat Rev 2014; 40(1): 12-21.

83 Zavros Y, Eaton KA, Kang W, Rathinavelu S, Katukuri V, Kao JY, *et al*. Chronic gastritis in the hypochlorhydric gastrin-deficient mouse progresses to adenocarcinoma. Oncogene 2005; 24(14): 2354-66.

84 Schumacher MA, Donnelly JM, Engevik AC, Xiao C, Yang L, Kenny S, *et al*. Gastric Sonic Hedgehog acts as a macrophage chemoattractant during the immune response to *Helicobacter pylori*. Gastroenterology 2012; 142(5): 1150-9.e6.

85 Song Z, Yue W, Wei B, Wang N, Li T, Guan L, *et al*. Sonic hedgehog pathway is essential for maintenance of cancer stem–like cells in human gastric cancer. PLoS One 2011; 6(3): e17687.

86 Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, *et al*. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell 2011; 19(2): 257-72.

87 Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, *et al*. Gastric cancer originating from bone marrow–derived cells. Science 2004; 306(5701): 1568-71.

88 Marx J. Medicine. Bone marrow cells: the source of gastric cancer? Science 2004; 306(5701): 1455-7.

89 Cao H, Xu W, Qian H, Zhu W, Yan Y, Zhou H, *et al*. Mesenchymal stem cell–like cells derived from human gastric cancer tissues. Cancer Lett 2009; 274(1): 61-71.