DNA双链断裂修复途径的选择与调控

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摘要 DNA双链断裂(double strand break, DSB)是一种导致基因组不稳定性的高毒性损伤,可引起染色质畸变诱发癌症.真核生物中演化出多条保守的DSB损伤修复途径,其中最重要的修复途径是典型的非同源末端连接(classical non-homologous end joining, cNHEJ)和同源重组(homologous recombination, HR),这两种修复途径解决了细胞中大多数的DSBs.对于不同类型的DSBs,细胞可以审时度势地选择最优的修复途径,以最低的基因组突变风险进行修复.依赖于不断发展的细胞成像技术和分子生物学方法,精细的DSB修复途径选择机制正逐渐显露出来,其调控机制十分复杂而且精密.本文主要阐述了断裂末端结构、DNA末端切除程度、细胞周期、染色质环境、组蛋白修饰和RNA代谢等因素对DSB修复途径的影响,展现了DSB修复途径选择的多样性和灵活性.

关键词 DNA双链断裂, DSB修复途径选择, 末端切除, 同源重组, BRCA1, 53BP1

生物体内的基因组时刻都面临着内源和外源的威胁,每个细胞每天都要发生多达 10^5 次DNA损伤^[1].大部分损伤都比较容易修复,但DNA双链断裂(double strand breaks, DSBs)修复是非常复杂的,它也是最具毒性的一种损伤.DSB指DNA双螺旋中两条链磷酸-糖骨架在相同或靠近位置同时发生断裂.在后生动物中,某些情况下单个DSB就会造成细胞的死亡^[2].DSBs在哺乳动物细胞主要通过典型的非同源末端连接(classical non-homologous end joining, cNHEJ)和同源重组(homologous recombination, HR)进行修复^[3].另外,还存在退火依赖的修复途径,包括单链退火(single strand annealing, SSA)和替代末端连接(alternative end joining, alt-EJ)^[4,5].不同的修复途径具有不同的保真性和适应性.对于不同情况的DSBs,选择最优的修复途径

至关重要,否则将导致基因组的不稳定性^[6].DSB修复缺陷增加了罹患癌症的风险,涉及多种人类遗传病,包括共济失调毛细血管扩张(ataxia telangiectasia, AT)、奈梅亨断裂综合征(Nijmegen breakage syndrome, NBS)以及严重联合免疫缺陷(severe combined immune deficiencies, SCID)等^[7-9].本文主要阐述了不同DSBs修复通路的分子机制,解析了不同的细胞因素对于DSB修复途径选择的影响.

1 DSB修复途径概述

DSBs在体内存在单端(one-end)和双端(two-end)两种形式,单端DSBs往往是由于复制叉行进到DNA单链断裂(single strand break, SSB)处形成的,诱导SSB损

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伤或者抑制SSB修复的药物都会导致复制相关的DSBs. 由于第二个末端的缺失, 单端DSBs倾向于进行HR修复^[10]. 本文主要介绍双端DNA断裂, DSB双端断裂修复途径的分类一直存在争议, 本文主要根据末端切除(resection)程度、是否依赖同源模板或DNA链退火将修复途径分为三类: cNHEJ、HR和依赖于退火的修复途径(图1). 其中, 将末端直接连接的cNHEJ是最简单的修复方式, 不需要末端切除, 也不依赖或极少依赖于同源序列. 另外两类修复方式则都以末端切除为起始, HR在末端切除后需要重组酶RAD51介导链入侵

过程, 同源模板往往选择复制后的姐妹染色单体. 依赖于退火的修复途径则是将暴露出的同源序列的两条单链进行退火从而进行断裂链的连接.

1.1 cNHEJ修复途径

cNHEJ虽然被叫做非同源末端连接, 但是近期实验表明, cNHEJ修复过程可能存在最多4 bp的微同源序列^[11]. 在真核生物中, cNHEJ发生在整个细胞周期^[12], 是快速的DSB修复途径^[13]. 在早期对哺乳动物细胞的观察中, 辐射损伤引起的DSBs通过cNHEJ途径

图 1 DSB修复途径总览. DSB发生后, Ku70-80会最先结合上来, 如果不发生末端切除, 会继而招募DNA-PKcs, ligase IV, XRCC4等cNHEJ核心因子介导cNHEJ修复途径. 如果末端发生MRN-CtIP介导的末端切除, 则会产生ssDNA抑制cNHEJ修复途径. 短程切除和长程切除产生的ssDNA可以通过链内退火进行修复, 分别被称为alt-EJ和SSA. 长距离切除产生的ssDNA也可以在BRCA2-PALB2-BRCA1复合体的帮助下和RAD51形成核蛋白纤维, 进行同源找寻和连入侵过程, 从而进入HR修复途径. HR途径又可以分为BIR, SDSA和DSBR

Figure 1 Overview of DSB repair pathways. The broken ends are first recognized and bound by Ku70-80. Without end resection, other cNHEJ core factors, such as DNA-PKcs, ligase IV, XRCC4, would be recruited to DSBs to mediate cNHEJ pathway. When MRN-CtIP-mediated resection occurs, the generated ssDNA will inhibit cNHEJ pathway. ssDNA from short-range and long-range resection can anneal in-strand to resolve the damages, termed Alt-EJ and SSA, respectively. ssDNA from long-range resection can also be bound by RAD51 to form nucleoprotein filament under the help of BRCA2-PALB2-BRCA1 complex. Nucleoprotein filament carry out homologous searching and strand invasion, promoting HR pathway. The HR pathway could be divided into BIR, SDSA and DSBR

快速地修复,其半衰期只有几分钟^[14]。虽然cNHEJ很少对细胞的生存造成问题,但是这种修复方式本质上容易产生修复差错,包括小的增添和缺失^[15]。cNHEJ修复途径也有小概率产生染色质易位(chromatin translocation),大约每 $2 \times 10^3 \sim 2 \times 10^4$ 个cNHEJ产物会发生一次^[16]。

cNHEJ的修复过程起始于套环状的Ku70-80异源二聚体围绕在DNA断裂末端。由于Ku70-80异源二聚体对DNA亲和性强且丰度很高, Ku往往是第一个结合在断裂末端的蛋白^[17]。Ku的结合保护了游离的DNA末端不被核酸酶消化,同时触发了cNHEJ其他组分的招募^[18],包括DNK-PCs(DNA-dependent protein kinase catalytic subunit), Artemis, DNA ligase IV和其他脚手架蛋白XRCC4(X-ray repair cross-complementing protein 4), XLF(XRCC4-like factor), PAXX(paralog of XRCC4 and XLF)和IFFO1(intermediate filament family orphan 1)等^[19-23]。DNA-PKcs对于DNA末端具有很高的亲和力,并且这种结合能力在Ku存在时会更加强烈^[24]。作为cNHEJ修复途径的核心激酶, DNA-PKcs可磷酸化自身和下游因子,扩大损伤修复信号。由于损伤的DNA末端往往不是平整的, cNHEJ有时也需要多种核酸酶和聚合酶对末端进行处理后才能进行连接^[17]。核酸酶Artemis和DNA-PKcs一同被招募到DSBs,在被DNA-PKcs活化后,其可以在单链DNA(single strand DNA, ssDNA)和双链DNA(double strand DNA, dsDNA)的边界处进行切割,这种处理涵盖了末端含有DNA悬臂(overhangs)、缺口(gaps)、环(loops)等多种情况^[25]。DNA ligase IV通过其C端两个BRCT结构域同时结合断裂端两侧的Ku70-80复合体,其XID结构域则可以和XRCC4相互作用^[11]。XRCC4-ligase IV的存在进一步稳定了断裂末端的桥接。近年来, XLF, PAXX和IFFO1等结构类似于XRCC4的蛋白也被鉴定出来,它们的存在促进了DNA ligase IV复合体的连接作用^[19,22,23]。研究表明, XRCC4和XLF及其同源蛋白可能以一种套筒状的结构围绕在断裂的DNA双螺旋外以稳定末端^[11],而IFFO1可以连接XRCC4和Lamin A/C,将DSB末端固定于细胞核骨架上,防止染色体易位,同时促进修复^[19]。

1.2 同源重组修复途径

不同于简单的cNHEJ修复途径, HR修复途径十分

复杂。由于需要同源模板进行修复, HR主要发生在S晚期和G2期,以利用复制后的姐妹染色单体作为模板。HR修复过程高度分层,需要连续多个过程^[26]。HR途径并不是单一的修复途径,根据其修复结果可分为不产生dHJ(double holiday junction)的合成依赖的链退火途径(synthesis-dependent strand annealing, SDSA)、断裂诱导复制途径(break-induced replication, BIR)和产生dHJ的典型HR途径(也叫作典型的DNA双链断裂修复(canonical DNA double-strand break repair, DSB))^[27]。

细胞进行HR修复时需要先对DNA末端进行切除,切除过程是HR的起始步骤,对于后续的修复过程也是必不可少的。切除过程由MRN-CtIP(MRE11-Rad50-Nbs1, CtBP-interacting protein)复合体起始,依赖于MRE11(meiotic recombination 11 homolog)的核酸内切酶活性和3'→5'核酸外切酶活性, DNA末端的5'端链被降解,形成游离的3'端单链悬臂^[18]。末端切除形成的单链DNA会迅速被单链结合蛋白RPA(replication factor A)包裹, RPA促进了核酸酶DNA2(DNA replication helicase/nuclease 2)介导的5'端链降解同时抑制了3'端链降解,从而保证了DNA末端切除的极性^[28]。同时, RPA也保护了ssDNA不受其他核酸酶降解,阻止ssDNA自身形成二级结构^[29]。但RPA的存在阻碍了HR后续的链入侵和同源搜索过程,因此RPA必须被重组蛋白RAD51(DNA repair protein RAD51 homolog 1)替换。由于RAD51对ssDNA亲和力远小于RPA,因此这种替换需要依赖重组介导蛋白。在酵母中, RAD51对RPA的替换由RAD52(DNA repair and recombination protein RAD52)进行,而在人类细胞中,这一功能主要由BRCA2(breast cancer susceptibility gene 2)承担。BRCA2缺陷的细胞响应电离辐射时,会呈现出RAD51聚集明显变少的表型^[30,31]。BRCA2有8个BRC结构域,每个结构域都可以独立和RAD51结合,前4个BRC结构域对RAD51亲和力高,促进RAD51在ssDNA上成核;后4个BRC结构域对RAD51亲和力低,促进RAD51纤维的延长^[18,32]。BRCA2招募到DSBs依赖于PALB2(partner and localizer of BRCA2)和BRCA1-BARD1(breast cancer susceptibility gene 1, BRCA1-associated RING domain protein 1)复合体(或RNF168(E3 ubiquitin-protein ligase RNF168))^[33,34]。ssDNA-RAD51核蛋白纤维突触一旦形成并稳定,就会进入HR下一个阶段——同源搜索。虽然目前对同源搜索的具体机制并不

是很清楚,但是这一过程可能依赖ssDNA-RAD51核蛋白纤维突触与不同的DNA双链进行多次短暂接触,随机探测基因组^[35]。找到同源模板后,ssDNA-RAD51核蛋白纤维突触就会入侵并打开双链DNA,通过Watson-Crick配对结合入侵DNA的互补链,这时会形成独特的置换环(D-loop)结构,而被替换的ssDNA则会被RPA包裹防止D-loop的倒转。链入侵并配对后,入侵的3'端就会以被入侵链为模板进行复制延伸。如果入侵的DNA分子非常稳定,那么DNA的复制依赖于DNA聚合酶 δ 一直延续到染色体末端,这种修复途径被称为BIR^[36];如果入侵链复制延伸后从模板链中分离并重新和另一个断裂末端退火结合,这种修复途径被称为SDSA。在人类细胞中,BLM(bloom syndrome protein), RECQ1(ATP-dependent DNA helicase Q1), RTEL1(regulator of telomere elongation helicase 1)等解旋酶通过参与D-loop的分解促进SDSA^[37-39];如果第二个断裂末端通过退火或链入侵的方式被整合进D-loop,之后经过DNA的合成和连接会形成dHJ结构,使修复进入DSBR途径^[27]。D-loop的稳定性决定了修复导向SDSA或者DSBR, D-loop的解离导向SDSA修复途径,反之导向DSBR。dHJ可以发生依赖于核酸酶的切割(resolution)过程,可能产生交叉(crossover)的产物,这一过程由SLX1-SLX4(structure-specific endonuclease subunit SLX1-4), MUS81-EME1(crossover junction endonuclease MUS81/crossover junction endonuclease EME1)或GEN1(Flap endonuclease GEN homolog 1)介导^[40,41];也可以发生依赖于分支迁移(branch migration)的解除(dissolution)过程,这一过程则由BTRR(BLM, topoisomerase III α , RMI1-RMI2)复合体介导,产生非交叉(noncrossover)的产物^[42]。

1.3 退火依赖的修复途径

类似于HR途径,退火依赖的修复途径也需要MRN-CtIP介导的切除。根据需要的同源序列长度分为SSA途径和alt-EJ途径,alt-EJ也被称作微同源介导的末端连接途径(microhomology-mediated end joining, MMEJ)。alt-EJ需要的同源序列较少,一般为2~20个碱基,而SSA则较长,一般大于25个碱基^[43]。SSA往往需要长范围的切除,依赖于重组酶RAD52直接将带有同源序列的两条单链进行退火,之后再通过ERCC1-XPB(DNA excision repair protein ERCC 1-DNA repair

endonuclease XPF)核酸酶复合体去除多余的ssDNA。由于这一过程会导致大量片端的丢失,因此SSA途径会严重威胁基因组的稳定性^[44]。alt-EJ常常被认为是一种候补的DSB修复方式,主要发生在重组不活跃,cNHEJ途径受损的细胞中^[45]。alt-EJ虽然也被叫做“末端连接”,但不依赖于cNHEJ的核心因子。alt-EJ途径需要通过PARP1(poly(ADP-ribose) polymerase 1)快速招募MRN复合体,引发末端切除,并且依赖于POL θ (polymerase θ)和DNA ligase III进行微同源序列的退火和连接^[46-48]。alt-EJ途径和SSA途径一样,本质上是倾向突变的,修复过程往往会产生缺失和增添。在cNHEJ受损的细胞中,易位染色体的连接处往往存在微同源序列,说明alt-EJ与染色质易位高度相关^[49]。

2 DSB末端切除程度决定修复途径的选择

正常细胞中多数DSBs都是由cNHEJ途径或者HR途径进行修复的,这两类修复途径在S/G2期存在明显的竞争关系。DNA末端切除过程对于DSB修复途径的选择十分重要,末端切除不仅影响Ku和断裂末端结合,抑制cNHEJ的发生,而且其产生的ssDNA也是所有HR修复途径的中间产物,可促进HR和退火依赖的修复方式。ssDNA也激活了DNA损伤应答(DNA damage response, DDR)中重要的分支——ATR(ataxia telangiectasia and Rad3-related protein)-ATRIP(ATR-interacting protein)介导的信号级联过程^[50]。末端切除的具体过程如下:在适当的细胞环境下,MRN-CtIP复合体解除CCAR2(cell cycle and apoptosis regulator 2)的限制^[51],在断裂末端依靠MRE11的核酸内切酶活性在5'链产生一个距末端最远达300 bp的缺刻(nick),之后MRE11或核酸酶EXD2(exonuclease 3'-5' domain-containing protein 2)再依靠3'→5'核酸外切酶活性将缺口延伸至断裂末端,完成短距离切除^[52,53]。形成的3' ssDNA会进一步由EXO1(exonuclease 1)或DNA2-BLM介导长距离的切除^[54,55]。DNA末端切除直接影响了DSB修复途径的选择,多种蛋白参与这一调控过程,但其中最具有代表性的就是53BP1(p53-binding protein1)和BRCA1^[56]。

2.1 53BP1抑制切除促进cNHEJ

在大约25年前,53BP1以p53结合蛋白的身份被首次发现^[57]。53BP1作为一个没有酶活性的蛋白,依靠其

大量DDR蛋白相互作用位点,作为募集平台参与DSB修复过程^[58,59]。53BP1虽然不是cNHEJ核心因子,但是参与了淋巴细胞长距离V(D)J重组和类别转换重组(class-switch recombination, CSR)等cNHEJ相关过程,并且53BP1直接抑制了末端切除,促进DSB修复转向cNHEJ^[60]。一项2009年的研究发现,当缺陷BRCA1的细胞同时失活53BP1后,细胞的生存能力得到了明显的回补^[61]。这一现象同样存在于多种模式细胞甚至小鼠胚胎模型中,BRCA1缺陷背景下,53BP1缺失可使细胞在衰老、染色体异常、药物敏感性等多种指标回复到较低水平^[61,62]。这些指标代表着细胞内HR的水平得到了明显回复。BRCA1是当时已知的促进HR途径的蛋白,因此,Bunting等人^[62]第一次提出了53BP1可以通过抑制依赖于CtIP的末端切除,从而促进cNHEJ修复途径的观点。这一观点在领域内迅速形成了一场找寻53BP1下游效应因子的热潮,RIF1(RAP1 interacting factor 1)^[63-66],PTIP(Pax transactivation domain-interacting protein)^[67]以及最近报道的Shieldin(SHLD1, SHLD2, SHLD3, Rev7)复合体^[68-74]被陆续发现。53BP1通过组蛋白的甲基化和泛素化标记(H4K20me2,

H2AK15ub, H3K79me)被招募到DSBs附近^[26],经过ATM(ataxia-telangiectasia mutated)激酶磷酸化激活后招募RIF1及下游的Shieldin复合体。Shieldin复合体中SHLD2具有三个OB-fold结构域,可以直接结合ssDNA,保护末端不被EXO1进一步切除^[75]。同时,Shieldin复合体也可继续招募CST(CTC1-STN1-TEN1)复合体和聚合酶 α -引物酶复合体(polymerase alpha-primase complex, Pol α -primase)。Pol α -primase可以通过填补(fill-in)的方式逆转切除的结果,从而促进cNHEJ的修复途径^[76](图2A)。而53BP1的另一个效应因子PTIP被证明可以抑制DNA2介导的长距离末端切除^[77]。除了抑制切除,53BP1也可以通过Shieldin复合体抑制泛素连接酶RNF168介导的RAD51的装载(loading)过程,从而从多个层面抑制HR,促进cNHEJ途径^[77]。

2.2 BRCA1促进末端切除和HR

BRCA1是早期发现的抑癌基因,其突变使女性罹患乳腺癌和卵巢癌的概率分别增加7倍和20倍,而且大部分BRCA1突变导致的乳腺癌是最为恶性的三阴乳腺癌^[78,79]。BRCA1强大的抑癌功能主要来自于其作为

图 2 53BP1和BRCA1拮抗调控末端切除。A: 53BP1-RIF1通过招募Shieldin复合体,抑制切除,促进cNHEJ修复途径; B: BRCA1移除53BP1对末端的屏蔽,促进切除和HR修复途径

Figure 2 53BP1 and BRCA1 antagonize the regulation of end resection. A: 53BP1-RIF1 recruits the shieldin complex to inhibit resection and promote the cNHEJ pathway; B: BRCA1 removes the barriers of 53BP1 and its effectors from the broken ends to facilitate resection and HR pathway

DSB修复途径调控蛋白, 促进切除过程、增强HR修复途径. BRCA1主要通过和BARD1形成异源二聚体发挥功能^[80]. 除了可以直接结合CtIP、促进MRN复合体介导的切除过程外, BRCA1促进切除最重要的方式是移除53BP1对末端切除的屏蔽作用(图2B). 在S/G2期, BRCA1的存在会明显缩小53BP1聚集的面积, 并且将53BP1外排到DSBs核心之外^[81]. BRCA1在S/G2期抑制53BP1依赖于染色质重塑酶SMARCA1(SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1)^[56]. BRCA1-BARD1二聚体介导组蛋白H2A第125、127、129位赖氨酸的泛素化, SMARCA1通过其泛素结构域CUE被招募到DSBs损伤位点^[80]. 在酵母中, SMARCA1的同源蛋白Fun30(ATP-dependent helicase FUN30)通过滑动核小体或者排离组蛋白H2A-H2B的方式影响53BP1在DSBs核心区域的定位, 在空间上允许具有切除功能的相关蛋白的进入, 从而促进切除过程^[82]. 此外, BRCA1还依靠多种途径抑制53BP1下游因子RIF1, 比如促进蛋白磷酸酶4C PP4C(serine/threonine-protein phosphatase 4 catalytic subunit)介导的53BP1去磷酸化过程^[83]和泛素连接酶UHRF1(E3 ubiquitin-protein ligase UHRF1)介导的RIF1泛素化过程^[84], 双向抑制了53BP1和RIF1的相互作用, 从而阻断53BP1下游信号. 除了增强末端切除, BRCA1-BARD1也可以促进RAD51的替换和装载, 从而支持HR修复过程^[79].

3 断裂末端的结构调控DSB修复途径选择

由于引起DSBs的诱因不同, 断裂末端的化学结构往往也不同. 只有少部份DSBs末端属于可以直接连接的平末端(clean), 这些DSBs通过快速的cNHEJ修复, 毒性也最小^[85]. 但大部分DSBs末端都含有特殊结构, 这些化学结构影响了DSB修复途径选择. Ku对平末端和含有少量ssDNA的末端都有较高的亲和力, 但不易与含有长ssDNA的末端结合^[86]. 末端ssDNA的长度影响了MRN复合体和Ku对末端的竞争, 进而影响了DSBs进行HR或cNHEJ. DSBs末端的化学结构经常被一些核酸酶处理, 包括Artemis、APLF(aprataxin and PNK-like factor)、解旋酶WRN(Werner syndrome ATP-dependent helicase)(含有外切酶活性)等, 一般情况下, 这些核酸酶的处理使末端产生极少的ssDNA, 从而促进

cNHEJ修复^[2]. 含有共价连接蛋白的末端是高毒性的, 这种损伤主要来自拓扑异构酶的异常. 对于这种损伤, 细胞可以通过TDP1/TDP2(tyrosyl-DNA phosphodiesterase 1/2)降解与DNA交联的蛋白, 进行倾向于cNHEJ的修复; 也可以通过MRN/CtIP的DNA剪切功能, 切除DNA-蛋白交联复合物, 使DSB修复倾向于HR或alt-EJ^[87,88].

4 细胞周期调控DSB修复途径选择

DSB修复途径选择受到细胞周期的调控. cNHEJ作为主要的修复途径在全细胞周期都存在; 而HR由于需要同源模板被严格地限定在S期和G2期, 其在G1期的发生会明显增加染色质易位的风险^[89]. 细胞周期调控主要依赖于细胞周期蛋白依赖性激酶(cyclin-dependent kinases, CDKs). CDKs的活性在G1期较低, 随着细胞周期进程逐渐升高, 直至分裂期达到顶峰^[90]. CDKs通过磷酸化CtIP多个位点促进切除, 使DSBs导向HR途径, 其中Thr817(可能为CDK2)和Ser327(主要为CDK1)的磷酸化分别对切除起始和CtIP-MRN-BRCA1复合体形成十分重要^[91,92]. CDKs也可以通过磷酸化EXO1和NBS1(Nijmegen breakage syndrome protein 1)促进切除过程^[93,94]. 除了直接促进切除, CDKs也会限制53BP1和ssDNA移位酶HELB(DNA helicase B)对末端切除的抑制^[90]. 在G1期, HELB在核内直接结合RPA, 抑制了EXO1或DNA2/BLM介导的长范围切除. 但随着细胞进入S期, CDK磷酸化HELB使其出核, 丧失抑制功能^[95,96]. 此外, 细胞周期蛋白也在促进HR方面发挥直接作用, 例如, cyclin D1可以结合RAD51促进其被招募到DSBs位点; cyclin A2在S期通过结合mRNA促进MRE11的表达量升高^[90].

在特殊的细胞周期发生DSBs会带来特殊的修复挑战, 影响修复途径的选择. S期复制叉的断裂或崩塌会形成独特的单端DSBs, 这种结构的修复只能依赖于BIR, 但是容易产生大量的突变^[36]. 在某些情况下, 有丝分裂期发生的DSBs虽然会发生DSB响应, 但是后续的cNHEJ修复过程会被阻断^[97].

5 染色体环境调控DSB修复途径选择

在真实的细胞环境中, 染色质的存在使DSBs的修

复变得十分困难。断裂末端周围染色质的结构特征和动态变化影响了DSB修复途径的选择^[98,99]。在高度凝聚、转录抑制的异染色质区的DSBs倾向于进行cNHEJ修复途径或alt-EJ修复途径;而在相对松散开放、转录活跃的常染色质区DSBs则偏好HR修复途径^[100]。目前发现,转录活跃区域的标记H3K36me3可以招募p75, p75促进了CtIP的招募从而促进HR修复途径^[101,102]。类似于H3K36me3,乙酰转移酶Tip60(60 kDa Tat-interactive protein)介导的H4K16ac也是转录活跃区域的标记,其可以促进BRCA1招募到组蛋白H4从而干扰53BP1的招募,抑制cNHEJ途径^[103]。但是有时在异染色质发生的DSBs会被移动到特殊的位置,继而发生切除,进行HR修复^[104,105]。在果蝇细胞中,异染色质区域的DSBs会在Smc5/6(structural maintenance of chromosomes protein 5/6)复合体的介导下移动到异染色质外周进行HR修复^[104]。类似地, rDNA中发生的DSBs有时也会被转移到核仁进行修复^[106]。这些DSBs的重新定位似乎依赖于SUMO化修饰。SUMO化过程除了促进DSBs的移动,可能还参与在DSBs原发位置对HR修复途径的屏蔽^[4,107]。

6 组蛋白修饰和染色质重塑酶调控DSB修复途径选择

组蛋白直接结合基因组DNA,其上分布着丰富的蛋白翻译后修饰靶点,复杂而精细地调控着多种DNA代谢活动,其中自然也包括DSBs损伤修复。组蛋白修饰过程往往发生在DDR上游,影响一系列修复蛋白的招募和活性。

H2AX是组蛋白H2A的变体,在哺乳动物中占全部H2A的2%~20%^[108]。细胞内感受到DSB发生后,PI-3Ks家族激酶会时间内磷酸化H2AX第139位丝氨酸(磷酸化状态被称为 γ H2AX), γ H2AX会沿着DSBs双向扩散损伤信号以招募损伤修复蛋白、细胞周期监测点蛋白和染色质重塑蛋白^[109,110]。H2AX的磷酸化过程对于HR和cNHEJ都是十分关键的。泛素化修饰也广泛存在于DSBs修复途径中,泛素连接酶RNF8/RNF168(E3 ubiquitin-protein ligase 8/168)和BMI1(B cell-specific moloney murine leukemia virus integration site 1)都会通过 γ H2AX信号被招募到损伤位点,分别介导对H2A/H2AX第13~15位赖氨酸和第119位赖氨酸的泛素化修

饰^[111,112]。这两种泛素化修饰的缺失都会同时影响53BP1和BRCA1的招募,导致HR和cNHEJ的受损^[113,114]。

组蛋白的乙酰化是调节染色质可进入性的关键因素。对组蛋白H3和H4氨基端尾上带正电的赖氨酸和精氨酸进行乙酰化,可以减弱其与带负电荷的DNA骨架的互作,从而使核小体松散便于修复蛋白的进入^[115]。其中,研究较多的包括H3K56和H4K16的乙酰化。在人类细胞中,这两种乙酰化过程分别由CBP(CREB-binding protein)/P300和MOF(male absent on the first)负责,这两种乙酰化标记都对DDR的正确响应十分关键^[110]。而去乙酰化酶HDAC1(histone deacetylase 1)或HDAC2的缺陷会造成H3K56和H4K16的持续乙酰化,抑制cNHEJ修复途径。这暗示进行cNHEJ修复时可能需要相对紧致的染色质阻止Ku的滑动^[116]。

除了组蛋白的翻译后修饰,染色质重塑过程也是调控DSB修复途径选择的重要方式^[115]。染色质重塑酶复合体依赖于ATP的水解移动、外排或者替换组蛋白从而影响损伤附近的染色质环境。染色质重塑酶可以根据结构功能和系统发育过程分为四或六个家族,本文依据经典分类将其分为INO80(inositol requiring 80),SWI/SNF(switching defective/sucrose nonfermenting),ISWI (imitation switch)和CHD(chromodomain, helicase, DNA binding)四个家族^[117,118]。INO80家族是最早被鉴定出来的重塑酶家族,成员包括INO80复合体、SWR1复合体、SRCAP(SNF2-related CREB-activator protein)复合体等^[26]。SWR1复合体通过 γ H2AX被招募到损伤位点后促进组蛋白H2AZ的替换。H2AZ的加入增强了染色质的开放程度,这也是BRCA1泛素化和招募所需要的。H2AZ的缺失会同时影响HR和cNHEJ的效率,迫使细胞进行alt-EJ途径^[119]。而有趣的是,INO80复合体则发挥正好相反的功能——移除染色质上的 γ H2AX和H2AZ。INO80复合体也被证明可以促进RAD51纤维的形成,其突变体会同时影响MRE11介导的切除和cNHEJ的效率^[120,121]。SWI/SNF家族的BAF(BRG1-associated factor)和PBAF(polybromo-associated BAF)之前都被发现涉及cNHEJ和HR修复通路。BRG1(transcription activator BRG1)是两种复合体中共同的ATP水解酶,BRG1的缺失造成RAD51对RPA的替换异常,导致HR效率的下降和致死性的提升^[122]。上文提到的

SMARCA1也是SWI/SNF家族的一员,在人类细胞中促进切除和重组.在ISWI家族中,重塑酶复合体的水解酶亚基SNF2H(sucrose nonfermenting protein 2 homolog)可以促进BRCA1的泛素化和招募,从而促进HR过程^[123].同时,SNF2H的招募依赖于RNF20/40(RING finger protein 20/40)介导的H2B的单泛素化,RNF20的缺失也会影响CtIP依赖的DNA切除过程^[124].关于CHD家族对DSBs修复途径选择的发现不是很多,但是最近CHD1(chromodomain helicase DNA binding protein 1)被发现可以促进CtIP的招募,其缺失会造成HR的受损而不影响cNHEJ^[125].CHD3/CHD4(chromodomain helicase DNA binding protein 3/4)促进了染色质变松,并且其招募需要另一个CHD家族复合体ALC1(amplified in liver cancer 1)的帮助^[126].

7 RNA代谢影响DSB修复途径选择

人类75%的基因组会被转录为ncRNAs(noncoding RNAs),这些ncRNAs涉及多种人类疾病和癌症,其中多类ncRNAs也被证明参与DSB修复途径的选择^[26,127].LncRNAs(long noncoding RNAs)作为ncRNAs中最大的一类,以多种形式参与DSBs修复.LINP1(lncRNA in non-homologous end joining pathway)在断裂位点会形成一种RNA支架结构,通过结合Ku70-80和DNA-PKcs促进cNHEJ^[128];DDSR1(DNA damage-sensitive RNA1)可以通过调控BRCA1-RAP80(BRCA1-A complex subunit RAP80)复合体招募从而促进末端切除,使DSBs导向HR途径^[129].除了lncRNAs,miRNAs(microRNAs)也参与DSB修复途径选择.miR-19和miR-335会抑制CtIP的水平,从而造成细胞HR缺陷和对损伤的过度敏感^[130,131].近期研究新发现了一类专门响应辐射损伤的ncRNAs,在哺乳动物中被称为DDRNs(Drosha- and Dicer-dependent small RNAs)^[132,133].DDRNs对于完全激活DDR是必要的,可促进MDC1和53BP1的招募^[133].

除了RNA自身,越来越多的RNA相关因子也被发现参与DSB修复和途径选择.PR19(pre-mRNA-processing factor 19)是一种涉及pre-mRNA剪接的泛素连接酶,其可以直接结合RPA招募到DSBs附近,通过泛

素化RPA激活ATR^[134].此外,PRP19可以通过调节BRCA1的水平促进HR修复途径^[135];而核外泌体蛋白EXOSC10(exosome component 10)可以促进RAD51高效装载,从而促进HR修复途径^[136].

8 总结和展望

近20年的研究使人们对DSB修复选择机制有了较清楚的认识.HR和cNHEJ作为主要的修复途径竞争性地在S/G2期修复DSBs,其中最关键的影响因素就是末端的切除程度.长距离的切除促进HR途径,相反受限的切除则偏好进行cNHEJ途径.除了依赖于时间(细胞周期)和空间(染色质环境)因素,断裂末端性质和RNA代谢也涉及其中.探讨BRCA1和53BP1如何拮抗地调节末端切除也是近年来研究的热点.虽然Shieldin复合体的发现使科学家第一次从分子层面解释了53BP1抑制切除的机制,但是该模型似乎还存在一些问题,例如为何一个抑制切除的复合体要结合在已经发生切除的ssDNA上?以及Shieldin复合体抑制切除是否真的依赖于CST-Pol α 介导的填补机制(因为Pol α 活性很差,一般只能连续合成20~25 nt^[137])?这些问题都等待着人们的发现.

对DSB修复途径的理解也有助于人们了解癌症发生机制,促进癌症靶向治疗.相比于正常细胞,癌症细胞会面对更高水平的DSBs压力.过度增殖的癌细胞依赖于DSBs的有效修复而存活.而癌症细胞往往存在遗传学的DDR缺陷,目前已经发现33种癌症(尤其是卵巢癌)的发生会伴随着HR的突变,因此合成致死(synthetic lethality)是目前应对某些癌症十分有效的治疗手段^[138].其中,最醒目的就是PARPi(poly(ADP-ribose) polymerase 1 inhibitors)对于BRCA1/2突变引起的乳腺癌和卵巢癌的治疗作用.目前在临床上已经有六种PARPi小分子药物,其中奥拉帕尼(Olaparib)、卢卡帕尼(Rucaparib)、尼拉帕尼(Niraparib)和他拉唑帕尼(Talazoparib)都已成功获批使用^[139].除了PARPi和BRCA1/2的组合,越来越多的合成致死组合也已经被发现,如RAD52和BRCA2^[140].相信未来随着对DSB修复机制的深入了解,人们将会开发出更多高效的癌症治疗方法.

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Pathway choice for DNA double strand break repair

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DNA double strand break (DSB) is a kind of highly toxic damage, causing genomic instability, chromatin aberration and cancer. A number of conserved DSB damage repair pathways have been found in eukaryotes, most notably classical non-homologous end joining (cNHEJ) and homologous recombination (HR). These two repair pathways resolve most DSBs in cells. For different types of DSBs, cells could wittily choose the optimal approach with the lowest risk for genomic instability. Relying on the developing cell imaging techniques and molecular biological methods, sophisticated mechanism of DSB repair pathway choice has been revealed. The regulation mechanism of pathway choice is complex and precise. In this review, the effect factors such as the structures of the broken terminals, the degree of DNA end resection, cell cycle, chromatin environment, histone modification and RNA metabolism on the DSB repair pathway choice are discussed, showing the diversity and flexibility of DSB repair pathway choice.

DNA double strand break, DSB repair pathway choice, end resection, homologous recombination, *BRCA1*, 53BP1

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徐冬一, 北京大学生命科学学院研究员. 分别于1999年和2005年获北京大学生物学学士学位和理学博士学位. 长期从事与DNA修复和癌症与衰老相关的多蛋白复合物研究. 带领研究团队围绕“DNA损伤应答调控网络”这一关键问题, 以“非同源末端连接(non-homologous end-joining, NHEJ)途径”为核心, 取得了一系列成果. 构建了完整的DNA修复蛋白相互作用网络, 由此揭示了细胞核骨架参与NHEJ的机制, 解析了DNA修复途径选择调控的分子机理. 作为通讯/共同通讯作者在*Nature Cell Biology*, *Molecular Cell*, *Proceedings of the National Academy of Sciences*, *eLife*, *Nature Communications*等高水平专业期刊上发表论文多篇, 为发展以合成致死为策略的抗肿瘤药物研发奠定了理论基础, 受到了国际同行的广泛认可和关注, 多次被亮点报道和引用.