HYPOTHESIS

Maintaining the unmethylated state

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Abstract

**Background:** A remarkable correspondence exists between the cytogenetic locations of the known fragile sites and frequently reported sites of hypermethylation. The best-known features of fragile sites are sequence motifs that are prone to the spontaneous formation of a non-B DNA structure. These facts, coupled with the known enzymological specificities of DNA methyltransferase 1 (DNMT1), the ATP-dependent and actin-dependent helicases, and the ten-eleven translocation (TET) dioxygenases, suggest that these enzymes are involved in an epigenetic cycle that maintains the unmethylated state at these sites by resolving non-B structure, preventing both the sequestration of DNA methyltransferases (DNMTs) and hypermethylation in normal cells.

**Presentation of the hypothesis:** The innate tendency of DNA sequences present at fragile sites to form non-B DNA structures results in de novo methylation of DNA at these sites that is held in check in normal cells by the action of ATP-dependent and actin-dependent helicases coupled with the action of TET dioxygenases. This constitutes a previously unrecognized epigenetic repair cycle in which spontaneously forming non-B DNA structures formed at fragile sites are methylated by DNMTs as they are removed by the action of ATP-dependent and actin-dependent helicases, with the resulting nascent methylation rendered non-transmissible by TET dioxygenases.

**Testing the hypothesis:** A strong prediction of the hypothesis is that knockdown of ATP-dependent and actin-dependent helicases will result in enhanced bisulfite sensitivity and hypermethylation at non-B structures in multiple fragile sites coupled with global hypomethylation.

**Implications of the hypothesis:** A key implication of the hypothesis is that helicases, like the lymphoid-specific helicase and alpha thalassemia/mental retardation syndrome X-linked helicase, passively promote accurate maintenance of DNA methylation by preventing the sequestration of DNMTs at sites of unrepaired non-B DNA structure. When helicase action is blocked due to mutation or downregulation of the respective genes, DNMTs stall at unrepaired non-B structures in fragile sites after methylating them and are unable to methylate other sites in the genome, resulting in hypermethylation at non-B DNA-forming sites, along with hypomethylation elsewhere.

**Background**

Our recent work on the mechanism of action of 2'-deoxyriboguanylurea (GuaUre-dR) [1], the primary breakdown product of 5-aza-2'-deoxycytidine (5azaC-dR) [2], coupled with work from multiple laboratories, as well as our own, on DNA methyltransferases (DNMTs) [3-8], the substrate specificity, mechanism of action and biological effects of helicases, such as the ERCC2, ATRX, HELLS and RecQ family of helicases [9-15], and the ten-eleven translocation (TET) dioxygenases [16-19], suggest that the mechanism responsible for most of the hypermethylation observed during carcinogenesis involves the breakdown of an epigenetic repair cycle that maintains the unmethylated state at and near the common fragile sites.

The classic examples of epigenetic downregulation in human cells and tissues are genes that are often silenced and hypermethylated during tumorigenesis. As demonstrated in Table 1, the vast majority of these genes reside at cytogenetic locations that define well-known fragile sites. This remarkable cytogenetic correspondence strongly suggests that hypermethylation, epigenetic downregulation and chromosomal fragility share common mechanistic features. The best-known feature of fragile sites is the presence of a sequence motif that is prone to the spontaneous formation of a non-B DNA structure. In addition to FRAXA [14], many other fragile sites have been shown to
Table 1 Hypermethylation at known fragile sites

| Gene   | Gene location | Fragile site | Fragile site type | Fragile site location | Methylation reference |
|--------|---------------|--------------|-------------------|------------------------|------------------------|
| RUNX3  | 1p36          | FRA1A        | Aph, C            | 1p36                   | [24]                   |
| ARHI   | 1p31          | FRA1C        | Aph, C            | 1p31.2                 | [25]                   |
| PAR1   | 1p22.1        | FRA1D        | Aph, C            | 1p22                   | [26]                   |
| S100A6 | 1q21          | FRA1F        | Aph, C            | 1q21                   | [27]                   |
| PTGS2  | 1q25.2-25.3   | FRA1G        | Aph, C            | 1q25.1                 |                        |
| DISC1  | 1q42.1        | FRA1H        | SazaC-R, C        | 1q42                   | [30]                   |
| GLUL   | 1q31          | FRA1K        | Aph, C            | 1q31                   | [31]                   |
| CCA2   | 1p31          | FRA1L        | Aph, C            | 1p31                   | [32]                   |
| MIR137 | 1p21.3        | FRA1M        | Fol, R            | 1p21.3                 | [33]                   |
| -      | -             | FRA2A        | Fol, R            | 2q11.2                 | -                      |
| BCL2L11| 2q13          | FRA2B        | Fol, R            | 2q13                   | [34]                   |
| MSH6   | 2p16          | FRA2D        | Aph, C            | 2p16.2                 | [35]                   |
| DOK1   | 2p13          | FRA2E        | Aph, C            | 2p13                   | [36]                   |
| RASSF1 | 3p21.3        | -            | -                 | -                      | [37]                   |
| MLH1   | 3p21.3        | -            | -                 | -                      | [38]                   |
| VH1    | 3p25.3        | -            | -                 | -                      | [39]                   |
| LRP1B  | 2q21.2        | FRA2F        | Aph, C            | 2q21.3                 | [40]                   |
| HOXD1  | 2q31.1        | FRA2G        | Aph, C            | 2q31                   | [41]                   |
| -      | -             | FRA2H        | Aph, C            | 2q32.1                 | -                      |
| FLIP   | 2q33-q34      | FRA2I        | Aph, C            | 2q33                   | [42]                   |
| KIF1A  | 2q37.3        | FRA2J        | Aph, C            | 2q37.3                 | [43]                   |
| ZEB2   | 2q22.3        | FRA2K        | Fol, R            | 2q22.3                 | [44]                   |
| RARB   | 3p24.2        | FRA3A        | Aph, C            | 3p24.2                 | [45]                   |
| FHT    | 3p14.2        | FRA3B        | Aph, C            | 3p14.2                 | [46]                   |
| -      | -             | FRA3C        | Aph, C            | 3q27                   | -                      |
| RARRES1| 3q25.32       | FRA3D        | Aph, C            | 3q25                   | [47]                   |
| PTX3   | 3q25          | FRA3D        | Aph, C            | 3q25                   | [48]                   |
| -      | -             | FRA4A        | BrdU, C           | 3q25                   | -                      |
| PDGFRA | 4q12          | FRA4B        | BrdU, C           | 4q12                   | [49]                   |
| SFRP2  | 4q31.3        | FRA4C        | Aph, C            | 4q31.1                 | [50]                   |
| SLIT2  | 4p15.2        | FRA4D        | Aph, C            | 4p15                   | [51]                   |
| -      | -             | FRA5A        | BrdU, C           | 5p13                   | -                      |
| -      | -             | FRA5B        | BrdU, C           | 5q15                   | -                      |
| NR3C1  | 5q31.3        | FRA5C        | Aph, C            | 5q31.1                 | [52]                   |
| GPR150 | 5q15          | FRA5D        | Aph, C            | 5q15                   | [53]                   |
| -      | -             | FRA5E        | Aph, C            | 5p14                   | -                      |
| APC    | 5q21-22       | FRA5F        | Aph, C            | 5q21                   | [54]                   |
| -      | -             | FRA5G        | Fol, R            | 5q35                   | -                      |
| Gene       | Gene location | Fragile site | Fragile site type | Fragile site location | Methylation reference |
|------------|---------------|--------------|-------------------|-----------------------|-----------------------|
| SCGB3A1    | 5q35.3        | FRA5G        | Fol, R            | 5q35                  | [55,56]               |
| -          | -             | FRA6A        | Fol, R            | 6p23                  | -                     |
| -          | -             | FRA6B        | Aph, C            | 6p25.1                | -                     |
| -          | -             | FRA6C        | Aph, C            | 6p22.2                | -                     |
| -          | -             | FRA6C        | Aph, C            | 6p22.2                | -                     |
| -          | -             | FRA6D        | BrdU, C           | 6q13                  | -                     |
| ESR1       | 6q25.1        | -            | -                 | -                     | [57]                  |
| -          | -             | FRA6E        | Aph, C            | 6q26                  | -                     |
| HACE1      | 6q21          | FRA6F        | Aph, C            | 6q21                  | [58]                  |
| -          | -             | FRA6G        | Aph, C            | 6q15                  | -                     |
| -          | -             | FRA7A        | Fol, R            | 7p11.2                | -                     |
| TWIST1     | 7p21.2        | -            | -                 | -                     | [56]                  |
| -          | -             | FRA7B        | Aph, C            | 7p22                  | -                     |
| -          | -             | FRA7C        | Aph, C            | 7p14.2                | -                     |
| IGFBP3     | 7p13-p12      | FRA7D        | Aph, C            | 7p13                  | [59]                  |
| HIC1       | 17p13.3       | FRA7D        | Aph, C            | 7p13                  | [60]                  |
| ABCB1      | 7q21.12       | FRA7E        | Aph, C            | 7q21.2                | [61]                  |
| TFF1       | 7q22          | FRA7F        | Aph, C            | 7q22                  | [62]                  |
| TES        | 7q31.2        | FRA7G        | Aph, C            | 7q31.2                | [63]                  |
| CFTR       | 7q31.2        | FRA7G        | Aph, C            | 7q31.2                | [64]                  |
| -          | -             | FRA7H        | Aph, C            | 7q32.3                | -                     |
| EN2        | 7q36          | FRA7I        | Aph, C            | 7q36                  | [65]                  |
| HSPB1      | 7q11.23       | FRA7I        | Aph, C            | 7q11                  | [66]                  |
| -          | -             | FRA8A        | Fol, R            | 8q22.3                | -                     |
| -          | -             | FRA8B        | Aph, C            | 8q22.1                | -                     |
| MYC        | 8q24.21       | FRA8C        | Aph, C            | 8q24.1                | [67]                  |
| MYC        | 8q24.21       | FRA8E        | Dmy, R            | 8q24.1                | [67]                  |
| CDKN2A     | 9p21          | FRA9A        | Fol, R            | 9p21                  | [68]                  |
| CDKN2B     | 9p21          | FRA9A        | Fol, R            | 9p21                  | [69]                  |
| BRN3P1     | 9q32-q33      | FRA9B        | Fol, R            | 9q32                  | [70]                  |
| CDKN2A     | 9p21          | FRA9C        | BrdU, R           | 9p21                  | [68]                  |
| CDKN2B     | 9p21          | FRA9C        | BrdU, R           | 9p21                  | [69]                  |
| DAPK1      | 9q21.33       | FRA9D        | Aph, C            | 9q22.1                | [71]                  |
|            |               | FRA9B        | Aph, C            | 9q32                  |                       |
| BARX1      | 9q12          | FRA9F        | SazaC-R, C        | 9q12                  | [72]                  |
| FRA10AC1   | 10q23.33      | FRA10A       | Fol, R            | 10q23.3               | [73]                  |
| PTEN       | 10q23.3       | FRA10A       | Fol, R            | 10q23.3               | [74]                  |
| -          | -             | FRA10B       | BrdU, R           | 10q25.2               |                       |
| EGR2       | 10q21.1       | FRA10C       | BrdU, R           | 10q21                 |                       |
| TYSND1     | 10q22.1       | FRA10E       | Aph, C            | 10q22.1               | [75]                  |
| -          | -             | FRA10E       | Aph, C            | 10q25.2               | [76]                  |
| MGMT       | 10q26         | FRA10F       | Aph, C            | 10q26.1               | [77]                  |

Table 1: Hypermethylation at known fragile sites (Continued)
| Gene   | Gene location | Fragile site | Fragile site type | Fragile site location | Methylation reference |
|--------|---------------|--------------|-------------------|-----------------------|-----------------------|
| RET    | 10q11.2       | FRA10G       | Aph, C            | 10q11.2               | [78]                  |
| MRPL48 | 11q13.4       | FRA11A       | Fol, R            | 11q13.3               | [79]                  |
| AMICA1 | 11q23.3       | FRA11B       | Fol, R            | 11q23.3               | [80]                  |
| CALCB  | 11p15.2       | FRA11C       | Aph, C            | 11p15.1               | [81]                  |
| HRAS   | 11p15.5       | FRA11C       | Aph, C            | 11p15.1               | [81]                  |
| MYOD1  | 11p15.4       | FRA11C       | Aph, C            | 11p15.1               | [64]                  |
|        | -             | FRA11D       | Aph, C            | 11p14.2               |                       |
| WT1    | 11p13         | FRA11E       | Aph, C            | 11p13                 | [82]                  |
| CD44   | 11p13         | FRA11E       | Aph, C            | 11p13                 | [82]                  |
|        | -             | FRA11F       | Aph, C            | 11p14.2               |                       |
| PGR    | 11q22-q23     | FRA11G       | Aph, C            | 11q23.3               | [84]                  |
| GSTP1  | 11q13         | FRA11H       | Aph, C            | 11q13                 | [85]                  |
| CCND2  | 12p13         | -            | -                 | -                     | [56]                  |
| CALCB  | 11p15.2-15.1  | FRA11I       | Aph, C            | 11p15.1               | [81]                  |
| HRAS   | 11p15.5       | FRA11I       | Aph, C            | 11p15.1               | [81]                  |
| MYOD1  | 11p15.4       | FRA11I       | Aph, C            | 11p15.1               | [64]                  |
|        | -             | FRA12A       | Fol, R            | 12q13.1               |                       |
| SLC6A15| 12q21.3       | FRA12B       | Aph, C            | 12q21.3               | [62]                  |
| CHFR   | 12q24.33      | FRA12C       | BrdU, R           | 12q24.2               | [86]                  |
|        | -             | FRA12D       | Fol, R            | 12q24.13              |                       |
| SELPLG | 12q24         | FRA12E       | Aph, C            | 12q24                 | [87]                  |
| BRCA2  | 13q12.3       | FRA13A       | Aph, C            | 13q12.2               | [35]                  |
| RB1    | 13q14.2       | -            | -                 | -                     | [88]                  |
| PCDH20 | 13q21.2       | FRA13B       | BrdU, C           | 13q21                 | [89]                  |
| PCDH20 | 13q21.2       | FRA13C       | Aph, C            | 13q21.2               | [89]                  |
| ZIC2   | 13q32         | FRA13D       | Aph, C            | 13q32                 | [90]                  |
|        | -             | FRA15A       | Aph, C            | 15q22                 |                       |
| ABCG6  | 16p13.1       | FRA16A       | Fol, R            | 16p13.11              | [64]                  |
| CDH1   | 16q22.1       | FRA16B       | Dmy, R            | 16q22.1               | [91]                  |
| CDH1   | 16q22.1       | FRA16C       | Aph, C            | 16q22.1               | [91]                  |
| CDH13  | 16q23.3       | FRA16D       | Aph, C            | 16q23.2               | [92]                  |
| WWOX   | 16q23.3-q24.1 | FRA16D       | Aph, C            | 16q23.2               | [93]                  |
| HIC1   | 17p13.3       | -            | -                 | -                     | [60]                  |
|        | -             | FRA17A       | Dmy, R            | 17p12                 |                       |
| BRCA1  | 17q21.31      | -            | -                 | -                     | [94]                  |
| SOX9   | 17q23         | FRA17B       | Aph, C            | 17q23.1               | [95]                  |
| CDH2   | 18q12.1       | FRA18A       | Aph, C            | 18q12.2               | [72]                  |
| SERPINF5 | 18q21.33    | FRA18B       | Aph, C            | 18q21.3               | [96]                  |
| BCL2   | 18q21.3       | FRA18B       | Aph, C            | 18q21.3               | [64]                  |
|        | -             | FRA18C       | Aph, C            | 18q22.2               |                       |
|        | -             | FRA19A       | SazaC-R, C        | 19q13                 |                       |
|        | -             | FRA19B       | Fol, R            | 19p13                 |                       |
|        | -             | FRA20A       | Fol, R            | 20p11.23              |                       |
|        | -             | FRA20B       | Aph, C            | 20p12.2               |                       |
harbor sequences, such as the CCG triplet repeat, which form hairpins, slippage intermediates (Figure 1A) and quadruplex structures. Non-B intermediates are known to be exceptional substrates for de novo methylation by DNA methyltransferase 1 (DNMT1) [6,7,20] either at its three-nucleotide recognition motif (Figure 1) within the repeat if it contains CG sites or at the same motif at CG sites flanking the non-B sequence if it does not. Consequently, even fragile sites that contain AT-rich sequences with high torsional flexibility and the potential for non-B DNA structure formation are subject to methylation in regions flanking the repeat. Other fragile sites that lack CG dimers, such as the Huntington’s disease CAG repeat, which can also form hairpins and slippage intermediates [7,21], appear to induce methylation at the flanking and other regions where CG dimers occur [7,22]; for a review, see Lukusa and Fryns [23].

Presentation of the hypothesis

The key components of the hypothesis, presented in Figure 1, are: 1) carcinogenesis-linked hypermethylation that occurs primarily at or near fragile sites as a result of the tendency of DNA sequences at these sites to form non-B structures; 2) methylation is applied de novo to these structures and their neighboring sequences not only by DNMT3A/3B but also by DNMT1; 3) during normal replication methylated non-B DNA structures are returned to the B form by ERCC2, ATRX, HELLS and RecQ helicases; 4) sequences that cannot be resolved by helicase action are removed by excision; 5) hydroxymethylation applied to the nascent methyl groups by the action of TET dioxygenases prevents sequences that are resolved by helicase action from undergoing maintenance methylation by DNMT1, regenerating the unmethylated state at these sites in normal cells (in this regard, it is important to recognize that resolution of these structures will result in hemimethylated DNA, and that hemimethylated DNA is the preferred substrate of TET1 dioxygenase [17]); and 6) in addition to DNA damage, carcinogenesis-linked dysfunction among the helicases results in hypermethylation at and near fragile sites, and hypomethylation elsewhere.

Testing the hypothesis

While the existing evidence for the proposed cycle is compelling, currently available experimental approaches permit several additional tests of the hypothesis. For example, transient knockdown by transfection-mediated expression of an ERCC2, ATRX, HELLS or RecQ helicase is predicted to result in a transient hypermethylation, coupled with an increase in local hydroxymethylation content at affected fragile sites. Stable knockdown is expected to result in both hypermethylation at affected fragile sites and global hypomethylation. In particular, the knockdown of the WRN helicase (REQL2) is predicted to result in hypermethylation of the FHIT gene at FRA3B [108], coupled with enhanced bisulfite sensitivity [109] of native DNA associated with the increased presence of non-B DNA structure at this site [109,110]. Existing studies on the effect of WRN mutations on methylation, for example, do not address early events at fragile sites, since they use cell lines that have been carried in culture or were isolated from adults bearing the WRN mutation [111,112]. Chromatin immunoprecipitation with antibodies to DNMT1 is expected to yield DNA that is enriched for fragile site sequences after helicase knockdown. Determining the levels of DNMT1 by immunoblotting after helicase knockdown would determine whether or not the removal of stalled DNMT1 involves proteolysis [113]. WRN knockdown coupled with DNMT1 knockdown is expected to produce enhanced bisulfite sensitivity [109,110] in the absence of hypermethylation, while enhanced bisulfite sensitivity after knockdown of DNMT1 alone would provide evidence for an obligatory role of methylation in non-B structure resolution.
Finally, as a test of the downstream portion of the cycle, overexpression of TET dioxygenases is expected to reduce de novo methylation at fragile sites caused by helicase knockdown, and knockdown of the dioxygenases should enhance de novo methylation at these same sites.

**Implications of the hypothesis**

The hypothesis is consistent with other suggestions for the genesis of hypermethylation [114,115]. Disruptions in the histone code might be expected to elicit fragile site formation, since exposure to carcinogens that damage DNA or block the histone modification processes, may also induce fragile sites. Alterations in DNA structure induced by miRNA (possibly via R-loop formation) could have similar effects at these sites. Moreover, the remarkable correspondence between sites of reported hypermethylation and fragile sites suggests that the mutational and epimutational base upon which natural selection can act during carcinogenesis is largely confined to these sites. Their tendency to adopt non-B DNA structures provides a compelling case for how they become available for natural selection.
Each of the tenets of the hypothesis is supported by cytogenetic, DNA methylation and enzymological evidence. Enzymological and biological evidence from our laboratory suggests that DNMTs have evolved to recognize non-B DNA structures, like those associated with FRAXA in fragile X-linked mental retardation, and FRA111/FRA111C in breast and prostate cancer [20,107,110], suggesting that DNMTs play an obligate role in the suppression of non-B DNA structures [116,117] along with associated repair systems. Given an obligate role for DNMTs in the suppression of non-B structure formation, the role of helicases in the process can be better understood. In general, deficiencies in helicases, such as ATRX, HELLS, BLM and WRN, have been shown to result in either global genomic demethylation [118,119], gene activation [10], or both global demethylation and gene activation. Two diametrically opposed interpretations of normal function of these helicases have been proposed. In one interpretation, they are viewed as actively promoting DNA methylation [118,120]. In the alternative interpretation, they are viewed as passively promoting normal DNA methylation by preventing the sequestration of DNMTs [107,117] at unresolved non-B structures [10]. The enzymological evidence supports the alternative interpretation. For example, the WRN helicase has been shown to resolve quadruplex DNA [15] and deficiency appears to result in the accumulation of non-B structures [10]. The evidence suggests that the DNMTs remain bound to non-B DNA sequences containing mispaired cytosines [107], oxidized bases [121] or DNA containing base analogs, such as deoxyuridine (dU) [122], 5azaC-dR or GuaUre-dR [1]. It follows, that in cases of helicase deficiency, DNMT sequestration at a site of hypermethylation will result in global hypomethylation, much like the effects of 5-azacytide (5azaC-R), 5azaC-dR and GuaUre-dR result in hypomethylation, since tightly bound DNMTs are unable to maintain normal methylation patterns. Moreover, this model (Figure 1) and the postulated obligatory role for DNMTs suggests that the cyogenetic overlap between 5azaC-R, 5azaC-dR and GuaUre-dR-induced fragile sites FRA1J and FRA9F, and the undercondensations observed in DNMT3B mutants [123] and knockouts [124], is the result of the complete titration of DNMT3B by non-B structures that remain unresolved and unrepaird after exposure to these compounds. The selective effect on DNMT3B as opposed to DNMT1 can be attributed to its low level of expression relative to DNMT1. Estimates from purification data [107] suggest that DNMT1 levels are in the order of several thousand copies per cell. Northern blotting suggests that the abundance of DNMT3B is ten to twentyfold below that of DNMT1 at a few hundred copies per cell [125]. The DNA footprint of DNMT1 is approximately 23 bp [126]. Thus, a single non-B structure involving even 1,000 bases of single-stranded DNA could sequester approximately 2% of the DNMT1 or 20% of the DNMT3B. Replication stress-inducing agents, such as aphidicolin or distamycin, can be expected to induce multiple non-B regions. During carcinogenesis, multiple rounds of sequential induction of fragile sites by replication stress and carcinogen action could result in global hypomethylation. Moreover, the shattering of metaphase that occurs at high concentrations of 5-azaCR contrasted with the confined induction of fragile sites at low concentration [127] is consistent with the idea that DNMT3B is knocked out at low concentration and DNMT1 at higher concentration, and that both are obligatorily involved in suppressing fragile sites. Finally, work with the TET dioxygenases [16-19] and the response of DNMTs to 5-hydroxymethylcytosine (hmC) strongly suggest that hydroxymethylation at repaired and methylated genes in fragile sites will act to restore the unmethylated active state of these genes (Figure 1B).

Abbreviations
5azaC-dR: 5-aza-2′-deoxycytidine; 5azaC-R: 5-azacytidine; bp: base pair; dU: deoxyuridine; DNMT: DNA methyltransferase; DNMT1: DNA methyltransferase 1; DNMT3: DNA methyltransferase 3; GuaUre-dR: 2′-deoxyriboguanylurea; hDNMT1: human DNA methyltransferase 1; HGCN: HUGO Gene Nomenclature Committee; hmC: 5-hydroxymethylcytosine; miRNA: microRNA; NMR: Nuclear magnetic resonance; ODN: Oligodeoxynucleotide; TET: Ten-eleven translocation.

Competing interests
The author declared that he has no competing interests.

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References
1. Lamparska K, Clark J, Babilonia G, Bedell V, Yip W, Smith SS: 2′-Deoxyriboguanylurea, the primary breakdown product of 5-aza-2′-deoxycytidine, is a mutagen, an epimutagen, an inhibitor of DNA methyltransferases and an inducer of 5-azacytidine-type fragile sites. Nucleic Acids Res 2012, 40:9788–9801.
2. Rogstad DK, Herring JL, Thiruvaruthu JA, Burdzy A, Perry CC, Neidigh JW, Sowers LC: Chemical decomposition of 5-aza-2′-deoxycytidine (Decitabine): kinetic analyses and identification of products by NMR, HPLC, and mass spectrometry. Chem Res Toxicol 2009, 22:1194–1204.
3. Smith SS, Hardy TA, Baker DJ: Human DNA (cytosine-5)methyltransferase selectively methylates duplex DNA containing mispairs. Nucleic Acids Res 1987, 15:6899–6916.
4. Smith SS, Kan JL, Baker DJ, Kaplan BE, Dernbeck P: Recognition of unusual DNA structures by human DNA (cytosine-5)methyltransferase. J Mol Biol 1991, 217:59–51.
5. Smith SS, Kaplan BE, Sowers LC, Newman EM: Mechanism of human methyl-directed DNA methyltransferase and the fidelity of cytosine methylation. Proc Natl Acad Sci U S A 1992, 89:4744–4748.
6. Smith SS, Laayoun A, Lingeman RG, Baker DJ, Riley J: Hypermethylation of telomere-like foldbacks at codon 12 of the human c-Ha-ras gene and the trinucleotide repeat of the FMR1 gene of fragile X. J Mol Biol 1994, 243:143–151.
7. Smith SS, Baker DJ: Stalling of human methyltransferase at single-strand conformers from the Huntington’s locus. Biochem Biophys Res Commun 1997, 234:73–78.
8. Smith SS, Baker DJ, Jardines LA: A G4-DNA/B-DNA junction at codon 12 of c-Ha-ras is actively and asymmetrically methylated by DNA(cytosine-5)methyltransferase. Biochem Biophys Res Commun 1989, 160:1397–1402.
biochemical recurrence among prostate cancer patients with clinically localized disease. Epigenetics 2006, 1:183–186.
30. Ghoshal K, Motwilala T, Claus R, Yan P, Katuy H, Datta J, Majumder S, Bai S, Majumder A, Huang T, Plass C, Jacob ST. HOXB13, a target of DNM738, is methylated at an upstream CpG island, and functions as a tumor suppressor in primary colorectal tumors. PLoS One 2010, 5:e10338.
31. Gavrichchik P, Shia A, O‘Leary K, Halev Y, Crook TR, Thompson AM, Lackner M, Lo NC, Schmid P. Epigenetic silencing of glucose synthetase (Glul) defines glucose depletion therapy. Cancer Res 2012, 72:p4-10-10. doi:10.1158/0008-5472.SABCS12-P4-06-10.
32. L’I. C, Cowell JK, Sossey-Alaou K: CLCA2 tumour suppressor gene in 1p31 is epigenetically regulated in breast cancer. Oncogene 2004, 23:1474–1480.
33. Kozaki K, Imoto I, Mogi S, Omura K, Inazawa J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. Cancer Res 2008, 68:2094–2105.
34. Fernandez SV, Huang Y, Snider KE, Zhou Y, Pogash TJ, Russo J: Expression and DNA methylation changes in human breast epithelial cells after bisphenol A exposure. Int J Oncol 2012, 41:369–377.
35. Moelans BC, Verschuuren-Maes AH, van Diest PJ: Frequent promoter hypermethylation of BRCA2, CDH13, MSH6, PAX5, PAX6 and WTI in ductal carcinoma in situ and invasive breast cancer. J Pathol 2011, 225:222–231.
36. Saulnier A, Vaisserie T, Yue J, Souda M, Malfroy A, Accardi R, Greveaux M, Sebastian S, Shahzad N, Gheit T, Hussain I, Torrente M, Maffini FA, Calabrese L, Chiesa F, Cuenin C, Shukla R, Fathallah I, Matos E, Daudt A, Kolmitz S, Wünsch-Filho V, Menezes AM, Curado MP, Zaridze D, Boffetta P, Brennan P, Tomlinson I, Maitra A, Heda S, Fong KM, Thunnissen F, Minna JD, Gazdar AF. Frequent promoter hypermethylation in primary carcinomas. Int J Cancer 2012, 130:2484–2494.
37. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeiffer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 2000, 25:315–319.
38. Herman JG, Mao J, Weisenburger DD, Hamilton SR, Kung AL, Cheadle A, Belinsky SA, Baylin SB, Issa JP. Frequent promoter hypermethylation in colorectal carcinoma. Int J Cancer 2000, 88:1122–1126.
39. Cavalli F, Shi A, O‘Leary K, Halev Y, Crook TR, Thompson AM, Lackner M, Lo NC, Schmid P. Epigenetic silencing of glucose synthetase (Glul) defines glucose depletion therapy. Cancer Res 2012, 72:p4-10-10. doi:10.1158/0008-5472.SABCS12-P4-06-10.
40. L’I. C, Cowell JK, Sossey-Alaou K: CLCA2 tumour suppressor gene in 1p31 is epigenetically regulated in breast cancer. Oncogene 2004, 23:1474–1480.
41. Kozaki K, Imoto I, Mogi S, Omura K, Inazawa J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. Cancer Res 2008, 68:2094–2105.
42. van Noesel MM, van Bezouw S, Voûte PA, Herman JG, Pieters R, Versteeg R: Clustering of hypermethylated genes in neuroblastoma. Cancer Res 2003, 63:1744–1740.
65. Bennett LB, Schnabel JL, Kelchen JM, Taylor KH, Guo J, Arthur GL, Wang LJ, Bai Y, Bao ZS, Chen Y, Yan ZH, Zhang W, Zhang QG, Yu J, Zhu T, Wang Z, Zhang H, Qian Z, Xu H, Gao B, Wang W, Gu L, Meng J, Kim YH, Lee HC, Kim SY, Yeom YI, Ryu KJ, Min BH, Kim DH, Son HJ, Rhee PL, Kinoshita T, Nomoto S, Kodera Y, Koike M, Fujiwara M, Nakao A, Beggs AD, Jones A, Shepherd N, Arnaout A, Finlayson C, Abulafi AM, Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE, Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Kuerbitz SJ, Kinoshita T, Nomoto S, Kodera Y, Koike M, Fujiwara M, Nakao A, Beggs AD, Jones A, Shepherd N, Arnaout A, Finlayson C, Abulafi AM, Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE, Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Kuerbitz SJ, Kinoshita T, Nomoto S, Kodera Y, Koike M, Fujiwara M, Nakao A, Beggs AD, Jones A, Shepherd N, Arnaout A, Finlayson C, Abulafi AM, Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE, Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Kuerbitz SJ.
85. Lee WH, Morton RA, Epstein J, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Smith SS, Dignam JD, Lebovitz RM, Roeder RG: Supercoiling-dependent sequence specificity of mammalian DNA methyltransferase at single-strand conformers from a site of dynamic mutation. J Mol Biol 1996, 275:67–79.
86. Picozzi LA, Richieri P, Bignami M, Franchitto A: Werner syndrome helicase activity is essential in maintaining fragile site stability. J Cell Biol 2008, 180:305–314.
87. Kho MR, Baker DJ, Laayoun A, Smith SS: Stalling of human DNA (cytosine-5) methyltransferase at a break-strand conformer from a site of dynamic mutation. J Mol Biol 1996, 275:67–79.
88. Pirzio LM, Yonemura S, Ohgaki H: Promoter hypermethylation of the RB1 gene in glioblastomas. Cancer Res 2006, 66:4617–4626.
89. Pfister S, Schlaeger C, Mendrzyk F, Wittmann A, Benner A, Kulozik A, Scheurlen W, Radlwimmer B, Lichter P: Array-based profiling of reference-independent methylation status (aPHINES) identifies frequent promoter methylation and consecutive downregulation of ZIC2 in pediatric medulloblastoma. Nucleic Acids Res 2007, 35:5851.
90. Pimpinelli A, Celardo A, Poulsen J, Albrechtsen A, Grzenkowska I, Bignami M, Franchitto A, Palmieri C, Bignami M, Franchitto A, Palmieri C: Fragile sites in human chromosomes. J Cell Biol 1994, 126:859–869.
91. Pimpinelli A, Celardo A, Poulsen J, Albrechtsen A, Grzenkowska I, Bignami M, Franchitto A, Palmieri C, Bignami M, Franchitto A, Palmieri C: Fragile sites in human chromosomes. J Cell Biol 1994, 126:859–869.
92. Pimstones J, Estell M, Fiorentino D, Kajimura TM, Brown WT, Laird CD: Accurate transcription initiation by TBP requires a SWI2/SNF2-like protein. Mol Cell 2002, 9:379–388.
93. Pimstones J, Estell M, Fiorentino D, Kajimura TM, Brown WT, Laird CD: Accurate transcription initiation by TBP requires a SWI2/SNF2-like protein. Mol Cell 2002, 9:379–388.
94. Pimstones J, Estell M, Fiorentino D, Kajimura TM, Brown WT, Laird CD: Accurate transcription initiation by TBP requires a SWI2/SNF2-like protein. Mol Cell 2002, 9:379–388.
95. Pimstones J, Estell M, Fiorentino D, Kajimura TM, Brown WT, Laird CD: Accurate transcription initiation by TBP requires a SWI2/SNF2-like protein. Mol Cell 2002, 9:379–388.