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Letter to the Editor

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To the editor: It was with great interest we read the review article 'DNA methylation in breast and colorectal cancer' by Agrawal *et al* (2007; 20, 711–721) since the topic of aberrant methylation is most interesting and highly relevant for our research and diagnostic activity. However, we were surprised to find that this peer-reviewed article presented several statements that were inaccurate and misleading to the readers.

In specific we would like to draw attention to the following points.

Page 711, column one 'DNA methylation, unlike the other epigenetic changes, does not alter the nucleotide sequence.' This appears incorrect as no epigenetic changes by definition should alter nucleotide sequence.

Page 714–715. The discussion on the BRCA1 and methylation confuses the reader since it appears like the gene and the gene product is mixed up (underlined). 'Alterations in the breast cancer susceptibility gene product (BRCA) accounts for half of the inherited breast carcinomas.[77] Its methylation is observed in breast and ovarian cancers, but not in colon and liver cancers, or leukemia indicating a tissue-specific process.[58] The frequency of methylation of this gene product is 38.5% in sporadic breast cancer.'[60] Our interpretation of underlying research articles is that it is the BRCA1 gene that is methylated, not the gene product. Another point is that the frequency of methylation of *BRCA1* gene in ref [60] was 9.1% (not 38.5%) Birgisdottir *et al* (2006). Breast Cancer Res. **8**:R38.

Furthermore, the follow-up sentences are unclear and mix up the concept of gene and gene product. 'Patients with a HER2/Neu-positive tumor indicate a highly aggressive breast cancer that requires special treatment, because it is amplified in 30% of invasive breast carcinomas.[55] DNA methylation is prevalent in the highly aggressive HER2/Neu-positive breast cancers; this gene is amplified in 30% of the cancers.[55] Increased aberrant methylation of steroid receptor genes and glycoproteins, such as progesterone receptor and E-cadherin, respectively, are associated with Her2/Neu-positive cancers'. We will also add to this section that the presence of Her2/Neu oncogene is not a prognostic factor for breast cancer. It is the Her2/Neu oncoprotein expression level and/or the Her2/Neu oncogene copy number that is prognostically relevant.

Page 716 the authors state that (errors underlined): 'Hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) accounts for 2–4% of all colorectal cancers and aberrant methylation of the mismatch repair genes, human mutL homolog 1 (hMLH1) or hMLH2, are the basis for the cancer.[115] …. High-level MSI sporadic colon cancer and HNPCC share histological features, proximal tumor location, and presence of tumor-infiltrating lymphocytes. They differ, however, in having widespread promoter hypermethylation of specific genes such as hMLH1 and BRAF.[112] We find this section misleading since (1) HNPCC is generally caused by mutations in *hMLH1*, *hPMS2*, *hMSH2* and *hMSH6*. Aberrant methylation ('epimutations') of *hMLH1* as a cause for HNPCC has been documented, but is rare. (2) Sporadic colon cancer differs from HNPCC by having activating mutations in *BRAF* codon 600 (not hypermethylation). Furthermore, Table 3 states hMLH1 as hypomethylated (should be hypermethylated).

The authors also state that on page 712 that hypomethylation results in X-chromosome inactivation. As this appears contradictory, at least to us, an explanation to the reader is warranted.

We hope that the authors will consider correcting the above points to maintain the high level of scientific precision of 'Modern Pathology' and avoiding confusing the reader. We will also add that we do by no means consider ourselves as experts in methylation and have only noted general points. Therefore, we urge the Editor to subject the article to a thorough new review before publishing a corrected version.

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In reply: We thank Dr Berg and Dr Steigen for their letter in response to our article on DNA methylation in breast and colorectal cancer.[1] We acknowledge the errors that were not caught during the proofreading of the paper. We agree that the term epigenetic refers to a heritable change in gene

expression that is mediated by mechanisms other than alterations in the primary nucleotide sequence. It is also correct that the frequency of methylation of the *BRCA1* gene is indeed 9.1% and we apologize for our mistake.[2] However, it does not change the conclusion regarding the functional importance of the methylation of *BRCA1*, the well-known breast cancer-related gene.[1] Indeed, it is the gene that is methylated and not the gene product.

In regard to the HNPCC, we referred to the findings of Gazzoli *et al*[3] who found a direct association between the methylation of the region of *MLH1* and the silencing of the gene in HNPCC. In a subset of sporadic colorectal cancers, increased microsatellite instability is caused by the inactivation of the mismatch repair gene *MLH1* due to promoter methylation. We agree that in HNPCC, which also shows increased microsatellite instability, mismatch repair inactivation results primarily from germline mutations. However, *MLH1* promoter methylation has also been found in a subset of HNPCC, and this is inversely associated with loss of heterozygosity.[4] Chan *et al*[5] reported a family with inheritance, in three successive generations, of germline allele-specific and mosaic hypermethylation of the *MSH2* gene, without evidence of DNA mismatch repair gene mutation. In this family, three siblings carrying the germline methylation developed early-onset colorectal or endometrial cancers, all with microsatellite instability and *MSH2* protein loss.[5] This suggests that promoter hypermethylation or point mutation could be responsible, at least in some cases, for the somatic loss of the mismatch repair genes, *MLH1* or *MLH2* in HNPCC.

In response to the request for an explanation of the X-chromosome inactivation and DNA hypomethylation, we refer to the published articles by Paning and Jaenisch[6] and by Beard *et al*[7] on the hypomethylation and silencing of X-linked genes.

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