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**Punctate MLH1 mismatch repair immunostaining in colorectal cancer**

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*Sir:* We read with interest the description by Niu *et al* of punctate immunostaining for the mismatch repair (MMR) protein MLH1 in a small series of cases of endometrial cancer, in association with complete loss of PMS2 immunostaining. (1) The authors conclude that unawareness of this rare pattern of aberrant MLH1 staining can result in erroneous reporting of such cases as isolated loss of PMS2 staining, interpreted as representing likely Lynch syndrome due to germline mutation, whereas in fact somatic hypermethylation of the *MLH1* promoter is the likely underlying molecular mechanism leading to defective mismatch repair in most such

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cases. We have observed this phenomenon similarly in a series of colon cancers, to the best of our knowledge not previously described in peer-reviewed literature, and herein describe our series, which support the conclusions of Niu *et al.*

Within a population-representative series of 706 stage 2/3 colon cancers diagnosed in Northern Ireland from 2004-2008, MMR immunohistochemistry (IHC) applied to tissue microarrays sections revealed a punctate pattern of staining for MLH1 (Roche-Ventana anti-MLH1 M1 mouse monoclonal antibody, prediluted, OptiView amplification) in nine cases, associated in all cases with loss of PMS2 immunostaining (Roche-Ventana anti-PMS2 EPR3947 rabbit monoclonal antibody, prediluted, OptiView amplification) (Figure 1). A further seven cases of isolated PMS2 loss were observed in this series, with normal MLH1 staining (Figure 2). The punctate MLH1 staining pattern was confirmed by repeating MMR IHC on whole sections, some tumours showing this pattern throughout but others demonstrating regions of loss of MLH1 staining. The punctate pattern of staining was not observed for any other MMR protein.

Comparing clinicopathological characteristics between these two groups, the mean patient age in the punctate MLH1 group was 73 (range 42-90) years, compared to 58 (range 39-74) years in the intact MLH1 group with isolated PMS2 loss. Of the punctate MLH1 cases, seven (78%) were female, compared to four (57%) in the intact MLH1 group. All punctate MLH1 cases and five (71%) of intact MLH1 cases originated in the proximal colon. Neither *MLH1* promoter methylation status nor germline MMR gene mutation status were available, but all 16 tumours were microsatellite instability (MSI)-high and four of the nine tumours with punctate MLH1 demonstrated somatic *BRAF* V600E mutation, compared to none in the intact MLH1 group.

A high proportion of colorectal cancer (CRC) cases with isolated PMS2 loss have underlying germline mutations in *PMS2* or *MLH1*, indicating Lynch syndrome. (2,3) However, PMS2 loss associated with absent or abnormal MLH1 staining is much more commonly a somatic rather than hereditary phenomenon. Somatic *BRAF* V600E mutation is strongly associated with sporadic MMR deficiency and effectively excludes underlying Lynch syndrome in the setting of a tumour with abnormal immunoexpression of MLH1 and PMS2. (4) Therefore cases of CRC demonstrating apparent isolated loss of PMS2 require careful scrutiny of MLH1 immunostaining to distinguish likely Lynch syndrome (true isolated loss of PMS2) from a likely somatic pathogenesis, characterised by punctate MLH1 staining, somatic *BRAF* mutation and characteristic clinical features of older age, female sex and right-sided predominance.

Niu *et al* have demonstrated, albeit in small numbers, an association in endometrial cancer of punctate MLH1 staining with MLH1 promoter hypermethylation. (1) While most cases of CRC with loss of PMS2 staining and punctate staining of MLH1 also likely represent somatic hypermethylation of the *MLH1* promoter, supported by the clinicopathological features of the patients in our series, we cannot exclude that this pattern may also be encountered in occasional Lynch syndrome-associated cancers. Of note, one such tumour in our series was from a 42-year-old female, whose tumour lacked somatic *BRAF* mutation. These features raise the possibility of underlying Lynch syndrome in this patient but are not diagnostic without germline mutation screening.

Given recent trends towards universal MMR status testing of all CRCs to detect Lynch syndrome, it is particularly important for pathologists to be aware of unusual variants of MMR immunostaining. (5) Germline non-truncating mutations in *MLH1* are long recognised, causing functional impairment of the MMR protein but with retained antigenicity and some protein expression. (6) It is possible that punctate MLH1 immunostaining may represent some variant

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of this phenomenon, although this has not been previously illustrated or described in previous reviews of MMR immunohistochemistry interpretation, to the best of our knowledge. (7)

In summary, awareness of this rare and unusual pattern of MLH1 immunostaining, observed in association with PMS2 loss, is important for accurate pathological reporting and clinical interpretation of MMR immunohistochemistry in CRC, as well as in endometrial cancer. We advise careful scrutiny of MLH1 IHC in any CRC demonstrating loss of PMS2 immunoreexpression. Should a punctate pattern of MLH1 immunostaining be observed, this should be reported as abnormal and further investigation for somatic *BRAF* mutation and/or *MLH1* promoter hypermethylation performed in consideration of the need for germline mutation screening to exclude Lynch syndrome.

**Figure 1.** Colon cancer demonstrating, within tumour nuclei, complete loss of PMS2 immunoreexpression (A) with punctate MLH1 expression (B). Note normal intense nuclear staining within adjacent internal control stromal and lymphoid cell populations which contrasts with the absent PMS2 staining and punctate MLH1 staining (immunoperoxidase, x400). This immunoprofile favours a sporadic pathogenesis to mismatch repair deficiency.

**Figure 2.** Colon cancer demonstrating, within tumour nuclei, complete loss of PMS2 immunoreexpression (A) with intact MLH1 expression (B) (immunoperoxidase, x400). Note the well-recognised heterogeneity of MLH1 staining which is comparable to that in surrounding control tissues. This immunoprofile favours underlying Lynch syndrome.

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