Oncogene (2013) **32,** 5331–5332

www.nature.com/onc

COMMENTARY

Genomic instability in pre-neoplastic colonic lesions

JA Shaw[1], TA Graham[2] and J Stebbing[3]

Genomic instability is a characteristic of most cancers and it is argued that genomic instability is a driving force for tumorigenesis. Data herein demonstrate that genomic instability, as evidenced by microsatellite instability (MSI) and promoter methylation of DNA mismatch repair genes, is common in individual glands of pre-malignant colorectal lesions and raises interesting questions about the role of MSI in the development of colorectal carcinoma.

Oncogene (2013) **32,** 5331–5332; doi:10.1038/onc.2013.21; published online 11 February 2013

In this issue of *Oncogene*, Beggs *et al.*[1] describe heterogeneous promoter methylation and microsatellite instability (MSI) in individual glands of pre-malignant colorectal lesions, demonstrating that genomic instability can originate in very early colonic dysplasia. Moreover, their data identify frequent within-tumour subclones marked by their MSI-status or particular patterns of promoter methylation, and raises the question of the prognostic significance of these clones.

Tumours that show microsatellite instability (MSI+), account for about 15% of all colorectal cancers (CRCs). Approximately 5% of CRCs occur in individuals with the HNPCC syndrome, who have inherited a germline mismatch repair gene mutation,[2–4] whereas the other 10% are sporadic and largely caused by hypermethylation of the *MLH1* gene promoter, leading to loss of the MLH1 protein.[5–7] These MSI+ CRCs, commonly located in the right side of the colon, are typically poorly differentiated, but have a relatively favourable prognosis,[3,4,8,9] thus warranting specific therapy. However, the evolution of MSI during the carcinogenic process has remained poorly understood.

Beggs *et al.*[1] have sought to address this by examining both MSI (identified by slippage at BAT25 and BAT 26) and promoter methylation of DNA mismatch repair genes (*MLH1, MSH2, MSH6, PMS2, MGMT and MLH3*) via methylation-specific MLPA in individual crypts extracted from colonic polyps. A total of 273 individual adenomatous crypts were isolated by microdissection from 91 sporadic polyps, which were an approximately equal mix of adenomatous and hyperplastic polyps. MSI was detected in about 21% of isolated glands, with roughly half of both adenomatous and hyperplastic polyps showing a mixture of MSI+ and MSS glands within a single polyp. MSI status was significantly associated with loss of HMLH1 expression, however, not all glands with HMLH1 loss were MSI+. Hyperplastic glands in particular showed methylation of the DNA repair genes, notably in *PMS2, MLH3* and *MSH3*, and multivariate logistic regression modelling showed that these methylation events are potentially significant in initiating MSI.

The mixture of MSI+ and MSS crypts within a single polyp is indicative of the time that the MSI+ phenotype evolved. A clonal ordering argument[10] implies that in the heterogeneous polyps MSI+ likely evolved as a subclone after the onset of neoplastic growth, whereas in the wholly MSI+ polyps the evolution of MSI likely occurred in the original adenomatous crypt prior to tumour growth. The timing of MSI development is a contentious issue;[11] the data of Beggs *et al.*[1] are indicative that is no single 'MSI pathway' to cancer and instead argue that MSI can develop at different points along the adenoma–carcinoma pathway in a manner that is largely independent of the degree of dysplasia.

Interestingly, the frequency of MSI described by Beggs *el al.*[1] in their gland-by-gland analysis is higher than has been reported previously. Beggs *et al.*[1] suggest that the previously reported lower rates of MSI (2–15%) may reflect that previous analyses have considered whole-polyp-DNA aggregates as opposed to single crypt lysates. In the cases where only a few glands are MSI+, the majority microsatellite stable DNA obscures the MSI+ DNA. Analysis at the individual gland level is therefore suggested to more accurately determine the true level of alteration and heterogeneity.

Given the differential diagnosis and treatment of MSI versus non-MSI cancers, a relevant question here is how the detection of a focal MSI+ clone within a benign adenoma relates to the development of a full-blown MSI+ cancer. The unprecedentedly high frequency of MSI+ found by Beggs *et al.*[1] gland-by-gland analysis would suggest that few MSI+ clones grow to routinely detectable size, or progress to form an MSI+ cancer. Thus, to assess the clinical implications of these data, it is critical to understand how and when MSI+ clones are established and how they can drive progression to malignancy.

Previous studies have identified hypermethylation of the *MLH1* gene promoter region in histological normal colonic mucosa from some CRC patients,[12] and have shown that this is a common age-related event, largely unrelated to the MSI status of the tumour.[13] Indeed, immunohistochemistry staining for MLH1 showed generally positive staining of normal crypts, but with patches or single crypts where staining was weak or absent. Together, these studies suggest a model in which a gradual age-related epigenetic drift at the *MLH1* promoter leads to the occurrence of an MSI phenotype.[13] Conceivably, this same epigenetic drift could also lead to demethylation of the promoter and the subsequent extinguishing of the MSI+ phenotype. Thus, the MSI phenotype may be only transitory and typically not contribute to future cancer evolution. The methodology employed by Beggs *et al.*[1] did not measure

[1]Department of Cancer Studies and Molecular Medicine, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, Leicester, UK; [2]Center for Evolution and Cancer, Helen Diller Family Comprehensive Cancer Center, Department of Surgery, University of California, San Francisco, CA, USA and [3]Division of Cancer, Imperial College School of Medicine, Hammersmith Hospital Campus, London, UK. Correspondence: Professor J Stebbing, Division of Cancer, Imperial College School of Medicine, Hammersmith Hospital Campus London, W12 0NN, UK.
E-mail: j.stebbing@imperial.ac.uk
Received 12 December 2012; accepted 20 December 2012; published online 11 February 2013

specific patterns of methylation at each locus; collecting these data could conceivably shed light on the dynamics of *MLH1* promoter methylation and the permanency of MSI+ clones.

MSI is usually assumed to accelerate carcinogenesis by increasing the rate at which selectively advantageous mutations are accrued. However, MSI can also potentially retard the growth of a clone by increasing the rate of deleterious mutation, and eventually lead to death of the clone.[14] Thus, it is conceivable that many of the focal MSI+ clones will go extinct due to their accrual of deleterious mutations; the clones that persist may be those which happen to accrue selectively advantageous mutations. The data of Beggs *et al.*[1] point to the need for a better understanding of the clonal evolution of MSI+ versus MSS clones within an adenoma in order to understand cancer aetiology. In summary, the present paper[1] has highlighted the importance of refining molecular studies, to analyse molecular alterations at the level of individual glands, including in normal tissue, to accurately investigate genomic instability and its role in driving the development of CRC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Beggs AD, Domingo E, Abulafi M, Hodgson SV, Tomlinson IPM. A study of genomic instability in early preneoplastic colonic lesions. *Oncogene* 2013; **32**: 5333–5337.
2 Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM *et al.* Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993; **104**: 1535–1549.
3 Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet* 1999; **36**: 801–818.
4 Salovaara R, Loukola A, Kristo P, Kaariainen H, Ahtola H, Eskelinen M *et al.* Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000; **18**: 2193–2200.
5 Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H *et al.* Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997; **57**: 808–811.
6 Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP *et al.* Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998; **95**: 6870–6875.
7 Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ *et al.* Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. *Cancer Res* 1998; **58**: 3455–3460.
8 Lothe RA, Peltomäki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L *et al.* Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res* 1993; **53**: 5849–5852.
9 Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994; **145**: 148–156.
10 Maley CC, Galipeau PC, Li X, Sanchez CA, Paulson TG, Blount PL *et al.* The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. *Cancer Res* 2004; **64**: 7629–7633.
11 Bodmer W, Bielas JH, Beckman RA. Genetic instability is not a requirement for tumor development. *Cancer Res* 2008; **68**: 3558–3560, discussion 3560–1.
12 Kuismanen SA, Holmberg MT, Salovaara R, Schweizer P, Aaltonen LA, de la Chapelle A *et al.* Epigenetic phenotypes distinguish microsatellite-stable and -unstable colorectal cancers. *Proc Natl Acad Sci USA* 1999; **96**: 12661–12666.
13 Nakagawa H, Nuovo GJ, Zervos EE, Martin Jr EW, Salovaara R, Aaltonen LA *et al.* Age-related hypermethylation of the 5′ region of MLH1 in normal colonic mucosa is associated with microsatellite-unstable colorectal cancer development. *Cancer Res* 2001; **61**: 6991–6995.
14 Datta RS, Gutteridge A, Swanton C, Maley CC, Graham TA. Modelling the evolution of genetic instability during tumour progression. *Evol Appl* 2013; **6**: 20–33.