Cancer

Minichromosome maintenance (MCM) proteins may be pre-cancer markers

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Aberrant overexpression of proteins called minichromosome maintenance (Mcm) proteins at the mucosal surface of dysplastic oesophageal squamous epithelium and Barrett's mucosa may indicate proliferation potential

Oesophageal cancer contributes about 3% of the cancer burden in the UK, 5% of cancer mortality, and the five year survival is a dismal 6% (www.crc.org.uk). There are two major types of oesophageal carcinoma, squamous cell carcinoma (SCC) and adenocarcinoma, each with different risk factors and epidemiologies. SCC arises from squamous cells lining the oesophagus and the geographical distribution of the disease shows wide variations, being virtually unknown in North Africa but common, for example, in eastern Turkey, Iraq, Iran, and northern China; high risk areas are generally associated with local food preservation practices that favour the generation of nitroso compounds from mould growing on pickled vegetables. In Western populations, heavy alcohol and cigarette consumption are well known risk factors for oesophageal SCC. Most adenocarcinomas on the other hand appear to arise from within areas of metaplasia known as Barrett's oesophagus, the metaplasia probably being caused by prolonged reflux of gastric acid and digestive enzymes (reflux oesophagitis). With the passage of time the epithelial lining becomes progressively more abnormal as it passes through a series of sequential steps that eventually result in the development of invasive adenocarcinoma. These steps include the development of glandular dysplasia, signalled by an increased nuclear:cytoplasmic ratio and loss of nuclear polarity within the cells lining the metaplastic glands: presumably morphological correlates of the underlying genetic alterations (commonly seen in neoplastic progression in other tissues) that are found in such glands.[1]

In the colon, the adenoma (by definition dysplastic)-carcinoma sequence is reasonably well understood, with large severely dysplastic villous adenomas having the most sinister reputation. Moreover, prophylactic removal of adenomatous polyps is the "norm", such is the seemingly inevitable progression of such lesions. The natural history of oesophageal dysplasia to invasive adenocarcinoma is not as clearly defined, and herein lies the problem. For example, two recent articles report somewhat different outcomes for severe dysplasia: Buttar *et al* found a cumulative cancer incidence at three years of 56% among patients initially presenting with diffuse (affecting >5 crypts or in multiple biopsies) high grade dysplasia,[2] whereas Schnell *et al* found a five year cumulative cancer incidence of only 9% among a group of 79 high grade dysplasia patients.[3] These apparent discrepancies may be partly attributable to the ways in which the pathology is "read"[4]; nevertheless, high grade dysplasia is an ominous finding. In this issue of *Gut*, Going *et al* report the aberrant overexpression of proteins called minichromosome maintenance (Mcm) proteins, along with Ki-67, at the mucosal surface of both dysplastic oesophageal squamous epithelium and dysplastic Barrett's mucosa **[see page 373]**.[5] As expression of Mcms was observed in almost all surface cells in high grade dysplasias, anti-Mcm antibodies may thus have a significant role in the recognition of this important precancerous state.

Initiation of DNA synthesis in eukaryotes is a complex multistep process involving the sequential loading of initiation factors into prereplicative complexes (pre-RCs) at replication origins, resulting in particular regions of chromatin being "licensed" for replication in the ensuing S phase.[6–9] The process begins with the binding of the origin recognition complex (ORC), and recruitment of Cdc6 and Mcm2–7 (minichromosome maintenance). The Mcms are highly conserved and were originally discovered and named as factors that supported minichromosome maintenance in yeast; the assembled Mcms are presumed to act as an enzymatically active (DNA unwinding) helicase.[10] As cells enter S phase, Cdc6 is released and other factors are added to the replication origin to initiate DNA replication; critically, Mcm proteins gradually dissociate from chromatin as the DNA is replicated. This negative regulation ensures that each region of DNA is replicated only once during a single cell cycle because replicated DNA lacks functional pre-RCs.

In non-neoplastic tissues, expression of Mcms is generally confined to the proliferative compartment in a pattern similar to conventional proliferation markers, such as Ki-67.[11 12] As such, conventional proliferation markers have not made a huge impact on tumour pathology diagnosis or cancer management, although they have been useful in areas such as the differential diagnosis of smooth muscle tumours, grading of soft tissue sarcomas, and prediction of metastases in thin melanomas. However, Mcms are not your average proliferation marker; even in some normal tissues such as premenopausal breast, Mcm expression far exceeds that of Ki-67 and may identify mammary gland progenitor cells.[12] Thus Mcms may be biomarkers of cells with replication potential (licensed to cycle!). In this respect, Mcms came to the attention of histopathologists/cytologists from work originating from the same stable as the current study of Going *et al*. In CIN of the ectocervix, where Ki-67 labelled approximately 10% of surface epithelial cells, by contrast Mcm5 and Cdc6 expression was observed in almost all surface cells. Such a huge discordance suggested that Mcm expression had utility as a marker of dysplasia and may identify that rare ("litigation") cell that might be missed by more conventional staining.[13] Likewise, in a variety of other dysplastic states (actinic keratosis, Bowen's disease, colonic tubulovillous adenomas) Mcms are more highly expressed than Ki-67, for example.[14] The present study by Going *et al* also suggests that Mcm expression may be a useful adjunct in identifying dysplasia in the oesophagus although curiously they did not observe the large discrepancy between Ki-67 and Mcm labelling seen in the cervix, epidermis, and colon. However, in common with previous studies,[14] Mcm expression was inversely correlated with tumour differentiation (keratinisation), supporting the notion that Mcms are indicative of proliferation potential.

The incidence of Barrett's oesophagus is increasing rapidly in the Western world and identification of high grade dysplasia within an area of Barrett's oesophagus has profound implications for the patient and gastroenterologist alike. If there is a method of surveillance for high grade dysplasia within patients who have Barrett's oesophagus that is practical, sensitive, and specific, and there is a defined treatment option which results in increased survival, then it would seem reasonable to pursue this surveillance programme. Although surveillance guidelines have been produced by the Practice

Parameters Committee of the American College of Gastroenterology,[15] other workers have found little enthusiasm for pursuing unselected Barrett's surveillance programmes.[16] This is where detection of Mcm expression may prove useful; Mcm expression along with other molecular markers may help in stratifying patients into low and high risk groups.[17] Furthermore, within an area of Barrett's oesophagus the endoscopist cannot biopsy all tissue and hence Mcm expression may allow detection of deregulated cell proliferation in a wider area than can be recognised on morphological criteria alone or perhaps allow brush cytology sampling of a wide area.

In summary, evaluation of Mcm expression probably represents an incremental step in the armamentarium for the detection of oesophageal dysplasia (particularly of high grade type) in Barrett's oesophagus, and thus may help facilitate the identification of a cohort of patients most at risk of disease progression.

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REFERENCES

1 **Jankowski JA**, Harrison RF, Perry I, *et al.* Barrett's metaplasia. *Lancet* 2000;**356**:2079–85.
2 **Buttar NS**, Wang KK, Sebo TJ, *et al.* Extent of high-grade dysplasia in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology* 2001;**120**:1630–9.
3 **Schnell TG**, Sontag SJ, Chejfec G, *et al.* Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 2001;**120**:1607–19.
4 **Spechler SJ**. Disputing dysplasia. *Gastroenterology* 2001;**120**:1864–8.
5 **Going JJ**, Keith WN, Neilson L, *et al.* Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. *Gut* 2002;**50**:373–7.
6 **Dutta A**, Bell SP. Initiation of DNA replication in eukaryotic cells. *Annu Rev Cell Dev Biol* 1997;**13**:293–332.
7 **Tye BK**. MCM proteins in DNA replication. *Annu Rev Biochem* 1999;**68**:649–86.
8 **Labib K**, Tercero JA, Diffley JF. Uninterrupted MCM2–7 function required for DNA replication fork progression. *Science* 2000;**288**:1643–7.
9 **Lei M**, Tye BK. Initiating DNA synthesis: from recruiting to activating the MCM complex. *J Cell Sci* 2001;**114**:1447–54.
10 **Labib K**, Diffley JF. Is the MCM2–7 complex the eukaryotic DNA replication fork helicase? *Curr Opin Genet Dev* 2001;**11**:64–70.
11 **Todorov IT**, Wemess BA, Wang HQ, *et al.* HsMCM2/BM28: a novel proliferation marker for human tumors and normal tissues. *Lab Invest* 1998;**78**:73–8.
12 **Stoeber K**, Tlsty TD, Happerfield L, *et al.* DNA replication licensing and human cell proliferation. *J Cell Sci* 2001;**114**:2027–41.
13 **Williams GH**, Romanowski P, Morris L, *et al.* Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci USA* 1998;**95**:14932–7.
14 **Freeman A**, Morris LS, Mills AD, *et al.* Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. *Clin Cancer Res* 1999;**5**:2121–32.
15 **Sampliner RE**. Practice guidelines on the diagnosis, surveillance, and therapy of Barrett's esophagus. The Practice Parameters Committee of the American College of Gastroenterology. *Am J Gastroenterol* 1998;**93**:1028–32.
16 **Macdonald CE**, Wicks AC, Playford RJ. Final results from 10 year cohort of patients undergoing surveillance for Barrett's oesophagus: observational study. *BMJ* 2000;**321**:1252–5.
17 **Bani-Hani K**, Martin IG, Hardie LJ, *et al.* Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *J Natl Cancer Inst* 2000;**92**:1316–21.

Cancer

Proximal and progressive: adenomas in HNPCC

D T Bishop

Characteristics of hereditary non-polyposis colorectal cancer adenomas

In this issue of *Gut*, Rijcken and colleagues[1] compare adenomas found in hereditary non-polyposis colorectal cancer (HNPCC) patients with those found in patients without a family history of colorectal cancer **[see page 382]**. HNPCC is an autosomal dominant syndrome associated with an increased risk of cancer at a number of anatomical sites but most noticeably of the bowel and endometrium. Some of the HNPCC adenomas examined in this study were derived from patients in families with known germline mutations in a DNA mismatch repair (MMR) gene (this topic has been reviewed recently in *Gut* by Wheeler and colleagues[2]). Colorectal carcinoma in patients with germline MMR mutations exhibit failure of DNA MMR as a result of loss of expression of both copies of the MMR gene. Failure of DNA repair implies that particular DNA sequences, if mutated somatically, are less likely to be corrected.[2]

There are three notable features of carcinomas arising as a result of HNPCC: (i) an excess of proximal lesions, (ii) an excess of mucinous and undifferentiated lesions, and (iii) evidence of rapid progression rates for some lesions.[3 4] Studies of HNPCC adenomas have been limited and therefore the study reported here is particularly welcome. The study focuses on the comparison of HNPCC adenomas and adenomas derived from subjects without a family history of colorectal cancer (termed "sporadic" in this publication). The authors reported an increased proportion of proximal HNPCC adenomas and that larger proximal HNPCC adenomas were more likely to be highly dysplastic than "sporadic" adenomas. Furthermore, HNPCC proximal adenomas were more likely to be dysplastic than HNPCC distal adenomas. These observations are therefore novel and are consistent with previous reports of increased numbers of proximal cancers in HNPCC by suggesting that the adenomas are more likely to be right sided and also that proximal HNPCC adenomas are more likely to progress than their distal counterparts.

Studies such as that of Rijcken *et al* are important for two distinct reasons. The first is that our information on the natural history of HNPCC is so limited, and in terms of evidence based approaches to clinical management and/or intervention for HNPCC patients, we need to improve our overall knowledge. There are still insignificant numbers of predisposed individuals identified (and those that are known are distributed over

many centres) and under regular surveillance to allow collection of such information and material. The second reason is that by making comparisons between HNPCC and "sporadic" neoplasia, we might learn more about the adenoma-carcinoma process. It is now over 10 years since Fearon and Vogelstein put forward a hypothesis on the development of colorectal neoplasia in terms of the known genetic and epigenetic changes.[5] While there is broad agreement about the general principles behind the model, relatively little is known of the details of tumour development within or outside the context of germline MMR mutations.[6] Research into the basis of the adenoma-carcinoma sequence is limited by the inability to examine the adenoma, other than at the time at which it is removed from the bowel, at some stage in its evolution. Inferences about longitudinal development must be made by examining the set of genetic changes and/or pathological characteristics present in different adenomas removed at different points along their development (and with differing and unknown potentials to become carcinomas) and attempting to interpret the differing patterns observed at those points in time. If all adenomas had the same sequence of events this would not present a problem. Inter-individual variation therefore restricts interpretation. Consistent differences between HNPCC and sporadic neoplasia may suggest fundamental differences along the pathway.

Adenomas arising in an individual with a germline MMR mutation and in a subject without such a mutation could differ because (i) the specific somatic mutations in the critical genes along the development pathway may be different, (ii) the order in which the critical genes are impacted may differ because some genes may be inherently more mutable than others because of their DNA sequence, or (iii) the critical genes may differ in the two processes. However, Homfray *et al* showed that the pattern of APC mutations did not differ depending on the MMR mutation status of the patient in whom the adenoma arose, suggesting the same role for APC in HNPCC adenomas as in sporadic adenomas.[7]

This study provides further evidence that loss of mismatch gene expression is an early step in the progression of adenomas arising in patients with an MMR germline mutation. Loukola and colleagues[8] found that analysis of microsatellite markers of tumour derived DNA was a useful screen for HNPCC. In this study, small HNPCC adenomas arising in patients with an MMR germline mutation did not show loss of expression of the MMR protein while larger adenomas had lost expression.

While the comparison of HNPCC and "sporadic" adenomas is the appropriate comparison to make, there are logistical constraints which limit the interpretation of some of the observations. For instance, screening practices differ for patients with germline mutations (regular colonoscopy) and those undergoing regular general population screening (sigmoidoscopy followed by colonoscopy if a positive finding in the distal colon). Such differences in screening methodology might well produce effects such as smaller adenomas in those undergoing regular screening and an excess of distal adenomas in the sporadic group, as indeed observed in this study. Such difficulties are inherent to this type of research and must be borne in mind.

This research is an important step towards furthering our understanding of the development of colorectal neoplasia. Comparisons of genetic changes and pathological characteristics of adenomas arising in those predisposed with those without overt predisposition may be interpretable because of our knowledge of MMR biology.

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REFERENCES

1 **Rijcken FEM** , Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. *Gut* 2002;**50**:382–6.
2 **Wheeler JM**, Bodmer WF, Mortensen NJ. DNA mismatch repair genes and colorectal cancer. *Gut* 2000;**47**:148–53.
3 **Jass JR**, Smyrk TC, Stewart SM, *et al.* Pathology of hereditary non-polyposis colorectal cancer. *Anticancer Res* 1994;**14**:1631–4.
4 **Jass JR**, Cottier DS, Jeevaratnam P, *et al.* Diagnostic use of microsatellite instability in hereditary non-polyposis colorectal cancer. *Lancet* 1995;**346**:1200–1.
5 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;**61**:759–67.
6 **Shibata D**, Aaltonen LA. Genetic predisposition and somatic diversification in tumor development and progression. *Adv Cancer Res* 2001;**80**:83–114.
7 **Homfray TF**, Cottrell SE, Ilyas M, *et al.* Defects in mismatch repair occur after APC mutations in the pathogenesis of sporadic colorectal tumours. *Hum Mutat* 1998;**11**:114–20.
8 **Loukola A**, Salovaara R, Kristo P, *et al.* Microsatellite instability in adenomas as a marker for hereditary nonpolyposis colorectal cancer. *Am J Pathol* 1999;**155**:1849–53.