MDM2 Expression in the Progression of Barrett's Oesophagus

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Sir: Barrett's oesophagus (BO) is the main precursor for oesophageal adenocarcinoma (OAC), and dysplasia arising within it is the standard marker used to predict risk of malignant progression. However, dysplasia diagnosis is subject to a significant amount of interobserver variation. Biomarkers may aid the diagnosis of dysplasia and may help predict the risk of progression to OAC. Abnormal expression of p53 can be reproducibly detected by immunohistochemistry (IHC) and it is an effective marker of risk of OAC[1]. There is however still a need for additional early stage markers.

MDM2 protein is a negative regulator of p53 which binds, ubiquitinates and inactivates it. However, MDM2 expression, particularly its relationship to p53 expression and mutation, in tumours appears to be variable and is little studied in BO and OAC[2].

We aimed to assess the expression of MDM2 as a potential marker of neoplastic progression in BO and investigate its relationships with p53 expression.

A total of 105 cases of BO were selected from the Barrett's dysplasia database at NUH from 2003 to 2016. Selected cases included a sample of ND, ID that progressed or did not progress to dysplasia or cancer as well as a sample of cases with LGD, HGD and OAC. Twelve cases were excluded due to lack of representative tissue. The remaining 93 cases were tested by IHC for MDM2 expression in formalin-fixed paraffin-embedded tissue samples. IHC was performed using MDM2 antibody (clone: ab3110, Abcam, Cambridge, UK) following citrate buffer antigen retrieval, at 1:2000 dilution in Leica antibody diluent (AR9352; Leica, Milton Keynes, UK). Samples were stained for p53 with D07 antibody (Dako, Ely, UK) using the Roche Ventana Ultra Benchmark system with automated heat mediated epitope retrieval. MDM2 and p53 immunostaining was assessed with H&E for each case. P53 staining was scored using a qualitiative approach based on the intensity in the atypical or dysplastic area relative to background non dysplastic mucosa, or for non-dysplastic cases in the area of greatest intensity. This approach is described in detail in Kaye et al. [3], and is similar to that used in Kaye et al.[4] and Kastelein et al.[5] The p53 staining was thus scored as negative (0), positive (1), absent (2), equivocal (3) and not representative (4). Positive expression and the "absent pattern" are regarded as reflecting abnormal p53 expression and likely p53 mutation[4]. The MDM2 nuclear staining was scored as absent (0), weak (1), moderate (2), strong (3) and not representative (4). The p53 staining was scored as negative (0), positive (1), absent (2), equivocal (3) and not representative (4). Similar to p53, this was assessed in atypical/dysplastic areas or in area of greatest intensity in non-dysplastic cases, and was based purely on intensity.

62% of p53 positive cases had strong MDM2 staining versus 34% with negative p53 (fishers exact test: p=0.002). There was a positive correlation (Spearman's rho value = 0.47, p<0.001) with increasing MDM2 staining as the grades of dysplasia increased (Table 1). However, there was no association (fishers exact test: p=1) between MDM2 staining intensity in ND/ID cases and progression to dysplasia or cancer (Table 2). Follow up times were however shorter in the MDM2 positive group (mean 15.5 months vs 36.5 months, P=0.04).

The positive association between abnormal p53 expression and MDM2 expression is counterintuitive since MDM2 is generally regarded as a negative regulator of p53. A previous study investigated the relationship between MDM2 and p53 mutation in OAC and BO specimens. This found, in line with established dogma, a negative association between TP53 mutation and MDM2 expression. However they did find, similar to our own study, that MDM2 expression tended to correlate with strength of p53 staining. A possible reason for

this discrepancy is that only part of the p53 gene was tested for mutations and it likely missed some mutations[6].

Another study in OAC by quantitative PCR and FISH showed 6% of OAC cases had definite MDM2 gene amplification and concurrent enhanced immunohistochemical expression of MDM2 protein but with no correlation to p53 mutation[7]. As this study was in advanced OAC, it is not known if this represented an early or late event in Barrett's carcinogenesis. The mechanism may be related to genomic doubling that has been recently proposed to be an important but late event in in the setting of BO[8].

Our data may be explained by the theory that MDM2 can be stabilised by mutant p53. Peng et al. found that mutant p53 resulted in the stabilisation and accumulation of MDM2 in a wide variety of epithelial and mesenchymal cancers[9]. Alternatively, our data may reflect the fact that MDM2 does have functions independent of p53; it may act in synergy with mutant p53 to drive dysplasia progression[10].

Thus we have unequivocally shown that increased MDM2 expression occurs more frequently with higher grades of dysplasia, alongside abnormal p53 expression. However, MDM2 expression is not a useful marker for predicting progression to OAC. Future research should continue to investigate whether MDM2 biologically is relevant in the development of OAC.

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Ethics

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Tables

Table 1. Expression of MDM2 in the progression of BO

	MDM2 Intensity				
	0	1	2	3	Total
No dysplasia	1	10	2	0	13
Indefinite	1	16	8	1	26
LGD	0	10	10	1	21
HGD	0	3	8	2	13
Carcinoma	1	0	5	3	9
Total	3	39	33	7	79

Spearman's rho value = 0.47, $p<0.001$

Table 2 Progression of non dysplastic and indefinite for dysplasia BO in relation to MDM staining

	MDM 2 Intensity		
Progressed	Weak	Strong	Total
No	16	7	23
Yes	12	4	16
	28	11	39

Fishers exact test: p=1

Figures

Figure 1: Barrett's oesophagus, no dysplasia (A) H&E; (B) p53 negative; (C) MDM2 score 1

Figure 2: Barrett's oesophagus, high-grade dysplasia (A) H&E; (B) p53-absent pattern; (C) MDM2 dysplastic glands score 3.