Recognition of the null staining pattern increases the utility of p53 IHC in Barrett's esophagus

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Abstract

Background: Mutation and/or deletion of p53, a cell cycle regulatory gene, is a promising biomarker for predicting the risk of neoplastic progression in Barrett's esophagus (BE). Although overexpression of p53 by immunohistochemistry (IHC) is a useful surrogate for point mutations, complete absence of p53 protein by IHC in neoplastic cells (null pattern) has recently been shown to be highly correlated with truncation and deletion mutations. This study was designed to evaluate p53 expression patterns in BE with and without dysplasia.

Design: 2,817 biopsies with a diagnosis of BE with/without dysplasia that also had a p53 IHC stain performed between Jan. 1, 2010 and Nov. 12, 2012 were identified in the files of Miraca Life Sciences. This included 139 with high-grade dysplasia (HGD), 274 with low-grade dysplasia (LGD), 214 indefinite for dysplasia (IND) and 2190 negative for dysplasia (ND). IHC stains were classified as: wild type (WT, 1-15% nuclear staining throughout); point mutation pattern (PMP, foci with >50% nuclear staining); and null mutation pattern (NMP, foci with complete absence of nuclear staining).

Results: Abnormal p53 IHC expression patterns were detected in 442/2,817 (15.7%) biopsies, including 372/442 (84.2%) with PMP, 57/442 (12.9%) with NMP, and 13/442 (2.9%) with both patterns in the same biopsy; 70 (15.4%) of the putative p53 mutations were NMP. The frequency of p53 mutation patterns increased with increasing grades of dysplasia (Figure 2). While both PMP and NMP were identified in cases with and (rarely) without dysplasia, NMP was disproportionately found in biopsies with HGD than LGD, IND, and ND (32/133, 24.1% vs. 38/309, 12.3%; p=0.0019). 133/139 (95.7%) of HGD had abnormal p53 IHC.

Conclusions: p53 null staining pattern is readily identifiable, and accounts for 15.4% of all p53 mutations detectable by IHC in BE in our series. Recognition of the NMP increases the sensitivity of detection of p53 IHC mutation patterns in BE, and the vast majority of biopsies with HGD were found to have abnormal p53 IHC. These results suggest that, due to historical lack of recognition of the null pattern, the utility of p53 IHC as an adjunctive diagnostic marker in BE biopsies may be underestimated.

Background

p53 is a cell cycle regulatory gene that is expressed in proliferating cells, and is involved in DNA damage-induced cell cycle arrest. p53 functions as a tumor suppressor gene in human cancer; p53 mutations result in loss of normal p53 function in tumor cells, and are present in about 90% of esophageal adenocarcinomas.

Importantly, in the setting of Barrett's esophagus (BE), p53 mutations have also been found in about 75% of high-grade dysplasia and about 25% of low-grade dysplasia. p53 mutation status is a promising biomarker, and p53 alterations predict an increased risk of progression to cancer.

The utility of p53 as a biomarker is hampered by technical challenges; mutation screening is not practical in small biopsy samples, and immunohistochemical (IHC) analysis is traditionally thought to detect only point mutations (which account for about 80% of all mutations). Recently, a second abnormal pattern of p53 expression has been recognized that is characterized by total absence of p53 expression. The frequency of this null expression pattern has not been well-characterized in BE and dysplasia. Furthermore, this pattern has previously been classified as "wild type", suggesting that the significance of p53 as a biomarker has been underestimated.

Methods

We hypothesized that recognition of the p53 null staining pattern would increase the sensitivity of IHC for the detection of p53 alterations.

The files of Miraca Life Sciences were searched and 2,817 biopsies were identified with a diagnosis of BE, with or without dysplasia, that also had a p53 stain performed between Jan. 1, 2010 and Nov. 12, 2012. The cases included 139 with high-grade dysplasia (HGD), 274 with low-grade dysplasia (LGD), 214 with changes indefinite for dysplasia (IND) and 2,190 negative for dysplasia (ND). p53 IHC was performed on routine formalin-fixed paraffin-embedded sections using clone DO-7 according to the manufacturer's instructions. All cases were stained at the time of sign-out, at the pathologist's discretion, as part of the routine diagnostic work-up of the case.

p53 staining was scored using the following standardized criteria established at Miraca Life Sciences in 2010: wild type pattern (WT, 2-3+ nuclear positivity in 1-15% of cells; Figure 1a), point mutation pattern (PMP, at least one crypt with 2-3+ nuclear positivity in >50% of cells; Figure 1b), and null mutation pattern (NMP, at least one crypt, including crypt base, with complete absence of nuclear staining; Figure 1c). In addition to assessment of positive and negative controls performed with each staining run, internal positivity of non-neoplastic cells (lymphocytes, squamous epithelium and non-intestinalized columnar epithelium) was evaluated for adequacy of staining in every biopsy.

Results

p53 IHC expression revealed abnormal patterns in 442/2,817 (15.7%) biopsies, including 372/442 (84.2%) with PMP, 57/442 (12.9%) with NMP, and 13/442 (2.9%) with both patterns in the same biopsy. 70/442 (15.4%) of the p53 abnormalities were NMP, representing an 18.8% (70/372) increase in the total number of abnormalities detected. The frequency of p53 mutation patterns correlated with increasing grades of dysplasia (Figure 2). While both PMP and NMP were identified in cases with and (rarely) without dysplasia, NMP was disproportionately found in biopsies with HGD compared to LGD, IND, and ND (32/133, 24.1% vs. 38/309, 12.3%; p=0.0019). 133/139 (95.7%) of HGD had abnormal p53 IHC.

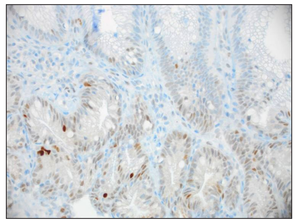

Figure 1a – p53 wild type pattern. There is 1+ positivity in about 30% of cells, 2+ positivity in about 3% of cells and 3+ positivity in about 1% of cells.

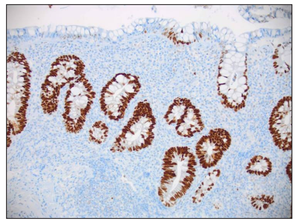

Figure 1b – p53 point mutation pattern. Crypt epithelium has 2+ positivity in about 20% of cells and 3+ positivity in about 80% of cells. Nuclear positivity extends focally onto the surface.

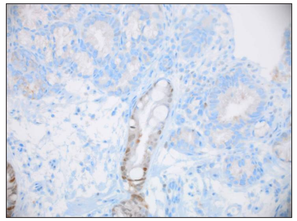

Figure 1c – p53 null mutation pattern. A single crypt in the center of the field has wild type positivity. Remaining crypts have no nuclear positivity.

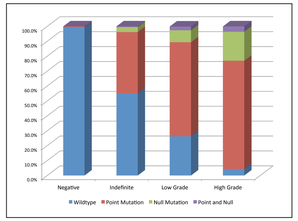

Figure 2 – Graphic representation of p53 status in Barrett's esophagus with and without dysplasia.

Study Highlights

- There are two distinct abnormal p53 IHC expression patterns in Barrett's esophagus and dysplasia: the point mutation pattern and the null mutation pattern.
- The p53 null mutation pattern comprises 15.4% of all detectable p53 IHC abnormalities.
- The p53 null mutation pattern makes up a greater proportion of detectable p53 abnormalities in high-grade dysplasia (compared to low-grade dysplasia, indefinite for dysplasia and negative for dysplasia).
- The vast majority of high-grade dysplasia (95.7%) have a detectable p53 IHC abnormality.

Conclusions

- Recognition of the p53 null mutation pattern increases the sensitivity of IHC for detection of p53 abnormalities by 18.8%.
- Prior studies that considered null mutation patterns as "wild type" have underestimated the frequency of p53 mutations and, therefore, the possible utility of p53 as a diagnostic aid and predictive biomarker.

References

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