The Molecular Pathology of Appendiceal Neoplasms

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Here we review the molecular pathological changes of appendiceal neoplasms, in part using the consensus terminology agreed by the Peritoneal Surface Oncology Group International (PSOGI)[1,2], with appendiceal neoplasms subcategorised as: hyperplastic polyps and serrated lesions (with and without dysplasia); appendiceal mucinous neoplasms (low and high grade); adenocarcinoma; goblet cell tumours; and neuroendocrine neoplasms. Compared with molecular pathological studies elsewhere in the gastrointestinal tract, the neoplasms of the appendix are less well investigated; however correlation of molecular pathology data with the revised histopathological subcategorisation provides improved understanding of the nature of these lesions, highlights some of the differences between them, and allows identification of actionable mutations that may help to guide future therapy.

Hyperplastic polyps and serrated lesions

Hyperplastic polyps are non-dysplastic and comprise elongated serrated crypts with increased numbers of goblet cells and mucin containing columnar cells. Serrated lesions are epithelial polyps in which crypts are dilated and the serrated morphology extends to the crypt bases. Serrated lesions may be non-dysplastic or show low grade dysplasia that may take the form of conventional adenoma-like dysplasia, serrated-type dysplasia or traditional serrated adenoma-like dysplasia. These neoplasms are usually incidental findings, although large lesions can be associated with acute appendicitis. Serrated lesions were historically thought

to be similar to those seen in the colon and rectum, which appear histologically comparable. However, molecular data from two studies reveal differences in the nature and frequency of mutations in appendiceal serrated lesions compared with those arising in the colon. Yantiss and colleagues[3] assessed a variety of genetic mutations in appendiceal serrated lesions with (n=23) and without (n=33) dysplasia and found similar mutation patterns in both. *BRAF* and *KRAS* mutations occurred at similar frequencies of around 30%. *TP53* mutations were not a significant feature, with only 1 mutation present (2%); and no Beta-Catenin mutations were found amongst this cohort. Mismatch repair protein and microsatellite instability studies were inconclusive. A subsequent study of appendiceal serrated lesions with and without dysplasia[4] found a low frequency of *BRAF* mutations, with only 5 mutations found in 126 lesions tested (4%). *KRAS* mutations were present in around 50%, with no differences found between non-dysplastic serrated, dysplastic serrated and non-serrated dysplastic types. These results contrast with the recognised serrated neoplasia pathway in the colon and rectum in which up to 90% of serrated lesions demonstrate *BRAF* mutations[5].

Appendiceal mucinous neoplasms (LAMN and HAMN)

Appendiceal mucinous neoplasms are defined by the lack of an infiltrative pattern of invasion, although a "pushing" pattern of invasion occurs. The epithelial architecture varies, mostly typically a filiform / villous mucinous proliferation; while undulating, scalloped or attenuated monolayered appearances can also be present. The majority of these lesions show only mild cytological atypia and are classified as low-grade appendiceal mucinous neoplasms (LAMN). Lesions with a similar architectural appearance (including the lack of an infiltrative growth

pattern) but high grade cytological atypia and increased mitotic figures are termed high-grade appendiceal mucinous neoplasms (HAMN). The incidence of such lesions is very low.

Mucinous neoplasms and mucinous adenocarcinomas may give rise to pseudomyxoma peritonei (PMP), characterised by a progressive accumulation of mucinous ascites which may be acellular or contain neoplastic epithelial cells. Several studies[6-11] demonstrated clonality and an appendiceal origin for almost all PMP[1]. The molecular pathology of mucinous neoplasms has been studied in primary appendiceal LAMNs and also in PMP lesions with an appendiceal origin. The reliability of studies which describe PMP as the histological diagnosis rather than the appendiceal histology is difficult to assess as both non–invasive (mucinous neoplasms) and invasive (mucinous adenocarcinoma) neoplasms of the appendix can give rise to PMP. No specific data on the molecular pathology of HAMNs is found in the literature, so these lesions will not be considered further.

A high frequency of *KRAS* mutations has consistently been found in LAMNs, although frequencies vary. Nishikawa and colleagues[12] found *KRAS* activating mutations in 94% of 32 LAMNs, while Zauber et al.[13] found *KRAS* mutations in all 31 LAMNs studied in a cohort which included LAMNs and PMP lesions. A large next generation sequencing study by Borozanci and colleagues[14] identified 83% *KRAS* mutations in PMP lesions. Lower frequencies have been found by some studies with smaller cohorts: 53% by Liu et al.[15], and 27% by Hara et al.[16]. The overall frequency of *KRAS* mutations in LAMNs from a review of the literature was calculated as 78%.

GNAS mutations are found consistently in a substantial proportion of mucinous neoplasms of around half, in contrast with adenocarcinomas of the appendix, with 6 studies finding

frequencies of 35-63%[6,7,12,14,15,17], although very small cohort studies have found lower frequencies[16].

The few next generation sequencing studies of LAMNs which have been carried out have allowed the recognition of lower frequencies of mutations in other neoplasia genes, including *PIK3CA* in 5-11%[6,7,14,15], *SMAD4* in 14-17%[6,7,14], and *TP53* in 5-27%[6,7,16]. p53 protein was overexpressed in 44%[8]. A single case with an *AKT1* mutation was found in the cohorts of 3 studies[6,7,15]. *APC* mutations were not found in any of 4 studies[6,7,13,14], but Liu and colleagues[15] found a small number of cases with mutated *APC.* Microsatellite instability has not been found in any study[7,13].

In summary, LAMNs are microsatellite stable and carry frequent *KRAS* and *GNAS* mutations with small numbers carrying a range of other mutations in cancer genes.

Adenocarcinoma

Appendiceal adenocarcinomas are defined by the presence of infiltrative invasion and include mucinous and signet ring subtypes[2]. Molecular studies of appendiceal adenocarcinomas have, to varying degrees, subcategorised cases by tumour grade or pattern (mucinous and/or signet ring adenocarcinomas). Most of these studies predate the more recent diagnostic guidance, giving rise to some inconsistencies in nomenclature. The molecular profiles differ significantly based on tumour subtype for some mutation loci.

KRAS mutations appear in around half of all adenocarcinomas. The largest NGS study by Borozanci and colleagues[14] comprised 442 adenocarcinomas, with *KRAS* mutations present in 55% of adenocarcinomas overall. 65% of the cases categorised as mucinous adenocarcinomas demonstrated *KRAS* mutation, compared to 47% of intestinal-type adenocarcinomas and only

7% of signet ring cell adenocarcinomas, implying different molecular profiles between the subtypes. A much smaller study[15] found *KRAS* mutations in 6 of 8 well differentiated adenocarcinomas associated with PMP and no mutations in 11 signet ring adenocarcinomas.

GNAS mutations are also present at a lower rate in adenocarcinomas than in LAMNs, with an overall combined mutation frequency of 16%, including 30% of mucinous adenocarcinomas and 6% of non-mucinous adenocarcinomas[12,15,17]. *BRAF* mutations were found in 8% of appendiceal adenocarcinomas[14], less frequently than in the colorectum, where mutations are present in 9-22% of tumours[18-20].

Mutations of the *TP53* gene appear to be present in approximately a quarter of appendiceal adenocarcinomas, with concordant frequencies of 24%-26% *TP53* mutations across subtypes found in two studies[15,21]. NGS analysis of mucinous, intestinal-type and signet ring adenocarcinoma demonstrated comparable *TP53* mutation frequencies of 24%, 32% and 15% respectively[14].

APC mutations also appear at significant frequencies in some subtypes: 7% in mucinous adenocarcinomas (n=317), 32% in intestinal-type adenocarcinomas (n=82) and 15% in signet ring cell adenocarcinomas (n=43). Liu et al.[15] found *APC* mutations in 27% of adenocarcinomas with goblet cells but none in well differentiated adenocarcinomas associated with PMP. However Jesinghaus and colleagues[21] found only 1 *APC* mutation within a colorectal type adenocarcinoma among 25 mixed adenocarcinomas of the appendix.

In contrast to mucinous neoplasms, which consistently show no evidence of microsatellite instability (MSI), small numbers of adenocarcinomas of the appendix have been found to

show high levels of microsatellite instability (MSI): 2 of 35 (6%) by Raghav et al.[22] and 3 of 96 cases (3%) by Taggart et al.[23]. In contrast, no adenocarcinomas with MSI were found by Jesinghaus et al.[21], 2018 (n=25), Borozanci et al.[14] (n=588), or Kabbani et al.[24] (n=30). The defective mismatch repair pathway of carcinogenesis appears less numerically important in the appendix than in the colorectum.

Goblet cell adenocarcinoma

Goblet cell adenocarcinomas (GCAs) are amphicrine tumours comprising cells with secretory phenotypes, including goblet cells, Paneth cells and endocrine cells, growing in tubules, small clusters, sheets or single infiltrative cells. Goblet cell adenocarcinomas have undergone significant reclassification over time, originally being classified as a carcinoid subtype, goblet cell carcinoid (GCC). The more aggressive phenotypes were given the designation 'adenocarcinoma ex goblet cell carcinoid' or 'mixed adeno-neuroendocrine carcinoma' in the 2010 WHO classification[25].

Recent molecular data now indicates these tumours are a distinctly different group from both neuroendocrine tumours (NETs) and adenocarcinomas (ACs)[21,26,27]. Sequencing studies analysed a series of GCCs and adenocarcinoma ex carcinoids (AC-EC), in addition to NETs and ACs[21,26] and found that GCC and AC-EC are best interpreted as a single morphomolecular entity, distinct from NETs and ACs. GCCs and AC-ECs demonstrated mutations in the WNT-signalling pathway genes *USP9X*, *NOTCH1*, *CTNNA1*, *CTNNB1*, and *TRRAP*; and in chromatin remodelling genes *ARID1A*, *ARID2*, *KDM6A*, and *KMT2D/MLL2*[21,26,27]. In contrast to the recognised tumourigenesis of colorectal adenocarcinomas, few or no mutations were found in *TP53*, *KRAS*, *SMAD4* and *APC* genes and all cases were microsatellite stable. This data

is in agreement with previous studies of GCCs that also found a lack of microsatellite instability[23,28] and of *KRAS* mutations[28-30]. P53 protein overexpression has not been found by some studies[29,31], although mutations in *TP53* in 4 of 16 cases (25%) were found by Ramnani et al.[30]. Allelic loss has been found at 18q (56%) 16q (38%) 11q (25%) and 6q (83%) in these tumours[26,29]. Some intestinal neuroendocrine tumours have also shown allelic changes at these and other loci (8q, 4p), but do not show the mutation pattern seen in GCCs and AC-ECs. Yozu et al.[32] presented a reclassification of this new combined entity as goblet cell adenocarcinoma (GCA) and proposed a grading system based on the overall proportion of tubular architecture.

Neuroendocrine neoplasms

Neuroendocrine lesions of the appendix are defined by the presence of cytoplasmic neurosecretory granules or microvesicles and comprise well differentiated neuroendocrine tumours (NETs), neuroendocrine carcinomas (NECs) and mixed neuroendocrine-non neuroendocrine tumours (MiNENs). The entity previously included in this category as goblet cell carcinoid has been recategorised as goblet cell adenocarcinoma, as previously discussed. Appendiceal NETs are of 3 subtypes, Enterochromaffin cell (EC), L-cell and tubular NET. Most are grade 1 and have good long-term survival. NECs are rare and no published molecular data is currently available. Tubular NETs may be a variant of L-cell NETs and tend to follow a benign course. MiNENs should contain by definition at least 30% each of neuroendocrine and non-neuroendocrine components. No specific molecular data was found relating to MiNENs, reflecting its recent description.

The molecular profile of NETs is not well established, although these tumours do not seem to harbour the mutational changes seen in other appendiceal or colorectal neoplasms. The large scale NGS study by Borazanci et al.[14] sequenced 81 neuroendocrine tumours and found an overall lower mutational rate than other the entities sequenced. *KRAS* mutations were found in 9%, *GNAS* in 3%, mutations in *SMAD4* in 13% and *TP53* mutations in 11%. These results contrast with those seen by Ramnani et al.[30] who used conventional and less sensitive sequencing of *KRAS* and *TP53* genes in 18 typical carcinoids and found no *KRAS* mutations but *TP53* mutations in 44%. 3 NETs were sequenced as a reference comparator in a further study[26], and no somatic coding mutations, copy number changes or pathogenic germline alterations were found.

	Major Molecular profile*
Serrated lesions	*BRAF* 4-30% *KRAS* 31-52%
LAMN	*KRAS* 78% *GNAS* 49% *PIK3CA* 5-11%; *SMAD4* 14-17%; *TP53* 2-27% Microsatellite stable
Adenocarcinoma	*KRAS* 53% *GNAS* 16% *TP53* 24-26% *APC* up to 32% with subtype Microsatellite instability in <6%
GCC	Loss of heterozygosity at 18q,16q, 11q and 6q *USP9X, NOTCH1, CTNNA1, CTNNB1, and TRRAP* *ARID1A, ARID2, KDM6A*, and *KMT2D/MLL2*
Neuroendocrine neoplasms	*TP53* 11-44%

Table 1. Overview of mutational profiles of appendiceal neoplasms (*mutation frequency in stated neoplasia gene, unless otherwise stated).

Discussion

Appendiceal neoplasms are not common, not extensively studied, and have undergone multiple reclassifications and nomenclature changes, all of which hamper attempts to collate the available molecular pathology data. In addition, published histopathological details of tumours that were molecularly analysed are often scant, and it is therefore likely that reported diagnostic categories include cases which would currently be subtyped differently. Despite this, mutational profiles have recently been instrumental in defining the current diagnostic criteria, for example, as defining goblet cell carcinomas as an entity in their own right rather than as a subtype of either neuroendocrine tumours or adenocarcinomas. The wealth of literature on the molecular pathology of colorectal neoplasms allows comparison with entities in the appendix, and many appendiceal lesions appear to be unique to this location. Adenocarcinoma of the appendix, although morphologically comparable to adenocarcinoma of the colorectum, appears to show a different molecular profile, with lower rates of microsatellite instability and *BRAF* mutations. Some recognised histopathological entities such as HAMN, hyperplastic polyps, neuroendocrine carcinomas and MiNEN are even less well molecularly profiled, usually due to rarity, but future molecular pathology data may help to further characterise these lesions.

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