



# The use of phenotype and genotype in the management of pT1 colorectal cancer

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Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality in the western world. Early cancers confined to the submucosa (stage pT1) are increasingly being diagnosed in countries with national bowel cancer screening programmes (1). Early cancers are generally associated with excellent outcomes. Many can be effectively treated with local excision, thus avoiding the need for a radical surgical resection and the associated morbidity and mortality. However, studies comparing local excision with major resectional surgery have shown an increased risk of local recurrence after local excision (2). This highlights the need for careful patient selection before the mesorectum is left in-situ. Around 8–14% of early cancers have lymph node metastases and there is a need to accurately identify which cases are likely to benefit from full resection (3-6). A number of high risk features for lymph node metastases are well recognised and routinely reported by pathologists e.g., size greater than 30 mm, poor differentiation, lymphatic invasion, venous invasion, involved resection margin and extension into the lower third of the submucosa. However, reporting of some of these features is subjective and they are often difficult to assess in practice, hence further phenotypic and genotypic biomarkers are required to accurately quantify the risk of nodal disease.

The retrospective study by Pai *et al.* aimed to identify phenotypic (i.e., histological) and genotypic (i.e., molecular) features that are associated with lymph node metastasis in early stage colorectal cancer (7). The authors specifically compared pT1 cancers with (n=28) and without (n=88)

lymph node metastases following surgical resection across two US centres between 2010 and 2014. They found a strong association between high tumour budding (defined as 5 or more buds per 0.95 mm<sup>2</sup>) and the presence of lymph node metastases. On univariate analysis, lymphatic invasion, submucosal invasion to a depth greater than 1,000 µm and high grade morphology were associated with an increased risk, however, only high tumour budding was independently associated with lymph node metastases on multivariate analysis. Not surprisingly, the presence of multiple high risk features led to an increased risk of nodal metastasis. Molecular analyses conducted included microsatellite instability testing (using polymerase chain reaction and immunohistochemistry) and a 50 gene mutation panel (using next generation sequencing). Out of 103 cases tested for microsatellite instability, 20% showed deficient mismatch repair, although no significant association with lymph node metastases was found. Mutational testing was only performed on a subset of 48 cases. Unsurprisingly, mutations involving the wnt and MAPK pathways and TP53 were the most prevalent in both groups. There was no association between lymph node metastases and any of the mutations, however, high tumour budding was positively related to TP53 mutations and negatively related to mutations in the mTOR pathway. Overall, patients with involved nodes in the study experienced a shorter time to disease recurrence when compared to patients with negative nodes.

The identification of tumour budding as a strong prognostic factor should come as no surprise. Tumour

budding is relatively consistently associated with metastatic nodal disease, although the risk is not quantified in most studies (6,8,9). Despite this, tumour budding is not utilised in routine practice in many countries due to the lack of a consistent and reproducible methodology. To address this, the International Tumor Budding Consensus Conference (ITBCC) was held in Bern, Switzerland in April 2016. Delegates from around the world, including Japan, Europe and North America, met and have subsequently recommended a standardised method of assessment, which should ideally now be built into national colorectal cancer pathology reporting guidelines (personal communication, Professor Phil Quirke, January 2017). This methodology will shortly be published in full but briefly consists of identifying budding (defined as a single cell or cluster of four cells or less) on a haematoxylin and eosin stained slide and counting the number of buds within a  $0.785 \text{ mm}^2$  area. The number of buds identified will then determine whether budding is classified as low, intermediate or high.

Lymphatic invasion and poor differentiation are frequently reported as being associated with nodal metastasis on multivariate analysis (5,9-11). Most national guidelines request that these features are routinely reported, however, there are difficulties in their assessment. Lymphatic invasion can sometime be challenging to distinguish from artefactual tissue retraction, and immunohistochemistry may be required to confirm an endothelial lining. Differentiation is subjective and it is hard to reach agreement on some cases between independent observers. There is clearly a need for developing novel reproducible quantitative biomarkers when assessing early colorectal cancer to assist with clinical decision making.

One such quantitative biomarker is the depth of submucosal tumour invasion, which has the advantage over Kikuchi levels of not requiring the entire submucosa (and in reality some muscularis propria to confirm that the entire submucosa is present) in the specimen (4). Of the studies where depth of invasion has been shown to predict nodal disease, the cut offs for high risk have varied between 1,000 and 2,000 micrometres (9,12). The measurement of depth of invasion has been shown to demonstrate good interobserver agreement in a study of 70 polyps reported by both gastrointestinal mono-specialists and pathologists who also report other specimen types (13).

Many pathology departments now have access to high resolution slide scanning and the ability to work with digital slides. This allows pathologists to measure certain quantitative features more accurately than with traditional

light microscopy. In addition, certain features such as tumour area can also be assessed on digital slides, which are impossible to assess down the microscope. A recent study from our own research group looked at various phenotypic features on digital slides from 207 pT1 cancers and the association with lymph node metastases (3). Tumours associated with nodal disease had both a greater width and greater area of submucosal invasion. The optimal cut offs for high risk were determined to be  $11.5 \text{ mm}$  and  $35 \text{ mm}^2$  respectively. On multivariate analysis, both were shown to be strong predictors of nodal metastases, with tumour area carrying the greatest weight. One potential explanation for this comes from further work in our group looking at the distribution of submucosal lymphatics (14). Using D2-40 immunohistochemistry and digital slides, lymphatics were demonstrated to be more numerous in the superficial third of the submucosa, with the deepest third containing the smallest number. Despite this, invasion into the deepest third is associated with the greatest risk of nodal disease (4). This suggests that it might be the overall area of tumour, especially in the superficial submucosa with its rich network of lymphatics, rather than the absolute depth of invasion that is important. Further work to confirm this hypothesis is now planned.

Molecular biomarkers are increasingly being used to subclassify cancers into prognostic groups and identify which patients might respond to specific therapies. In colorectal cancer, an international expert consortium has recently described four consensus molecular subtypes (CMS) of colorectal cancer, although around 13% of cases show features of more than one group (15). CMS1 shows frequent microsatellite instability and BRAF mutations, and accounts for approximately 14% of cases. Microsatellite instability is well recognised to be associated with a good prognosis with very few cases going on to develop metastatic disease (16). The study by Pai *et al.* showed a mismatch repair deficiency rate of 20% with slightly more nodal disease in the microsatellite unstable group. Whilst this was not statistically significant, the number of cases in the study was small and further investigation on a larger series is required to determine whether this biomarker could be used as a stratifier for formal resection in addition to its already accepted use as a strong prognostic marker. The CMS2 and CMS3 groups are associated with wnt/myc activation and KRAS mutations and account for approximately 50% of all cases between them. The final group, CMS4, shows the worst prognosis and is associated with the expression of stromal genes. Again using digital slides, our group has

shown that the proportion of the tumour area composed of stroma has a strong prognostic effect, with those stromal-rich tumours having a poorer prognosis (17). Whilst this study included both early and intermediate stage disease, there is no evidence to suggest that the proportion of stroma within pT1 disease does not have a similar prognostic effect. Further work specifically looking at the proportion of stroma in pT1 cancers is currently ongoing.

To conclude, the study by Pai *et al.* provides a good insight into how both phenotypic and genotypic biomarkers could be combined to stratify which patients with early colorectal cancer treated by local excision should be offered subsequent full resectional surgery. However, the study is small and further validation is required. A number of phenotypic biomarkers are already routinely reported by pathologists, however, several of these are subjective and poorly reproducible. Recent work has shown that budding should now be reported using the new ITBCC criteria and additional objective measurements including the depth and width of the invasive component should probably also be included. With the introduction of digital pathology, novel quantitative biomarkers including area of the cancer and proportion of stroma should be further explored. Finally, molecular markers are increasingly being used to classify colorectal cancer and whilst Pai *et al.* failed to show any clear association with nodal metastases, markers such as microsatellite instability are likely to be of importance. Further work is now urgently needed on larger western datasets to determine which of these markers are the most important and should be used in predictive models for the management of pT1 cancers. Pathologists are increasingly aware of the burden of responsibility placed on them to get this right, with the significant rates of postoperative mortality reported following major surgery when many cases with early disease would be successfully treated with local excision (18).

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