Tumor Budding in Colorectal Carcinoma: What, Why, and How

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Current Issues in Anatomic Pathology 2017

Outline

• Background and definition/terminology
• Why now?
• Practical considerations

Disclosures

• I have nothing to disclose

What IS tumor budding??
What is tumor budding?

Definitions

- **Tumor bud**
  - Most studies define as single tumor cells and tumor cell clusters composed of \( \leq 4 \) cells
  - Peritumoral vs. Intratumoral

- **Poorly differentiated clusters (PDC)**
  - Tumor clusters (composed of \( \geq 5 \) cells) and lacking glandular lumens
  - vs. Poorly differentiated carcinoma

- **Tumor grade ≠ Tumor budding**

Peritumoral vs. Intratumoral budding

Why is this a hot topic NOW??
Why is this a hot topic now?

- “Factors important to consider in making decisions about treatment” per AJCC guidelines (8th ed.)
  - Serum CEA levels
  - Tumor regression score in rectal carcinoma
  - Circumferential resection margin
  - Lymphovascular invasion (LVI) – small vessel versus venous
  - Perineural invasion
  - Microsatellite instability (MSI)
  - KRAS and NRAS mutation status
  - BRAF mutation

No tumor budding... YET

Why is this a hot topic now?

In the U.S., tumor budding is currently not a required element in the CAP cancer protocol for CRC (current as of January 2016)

Other organs where tumor budding is showing prognostic impact:
- Esophagus
- Breast
- Pancreas
- Lung

Importance recognized by:
- Union for International Cancer Control (UICC)
- Association of Directors of Anatomic and Surgical Pathology
- Included in guidelines for CRC screening, diagnosis, and treatment in Europe and Japan

Coming soon to a synoptic near you!

Tumor budding in colorectal carcinoma

- Early reports:
  - Imai, 1954: Postulated “sprouting” at invasive edge of carcinomas reflect a more rapid tumor growth rate
  - Hase et al., 1993: Prognostic value of tumor budding in colorectal cancer – “More severe budding was associated with worse outcome” (5-year and 10-year survival rates)
  - Ueno et al., 2002: “Because of its value as a prognostic indicator and its reproducibility, tumour ‘budding’ would be a good index to estimate the aggressiveness of rectal cancer.”
Tumor budding in biopsies

- Associated with:
  - Nodal and distant metastasis at time of resection
  - Non-response to neoadjuvant chemoradiotherapy
  - Poor survival outcome in rectal cancer patients

- INTRATUMORAL budding
  - Proposed cutoff of 6 tumor buds/HPF (400x)...

Tumor budding as a prognostic factor in resection specimens

- Stage I CRC (pT1/2 pN0 M0)
  - High-grade tumor budding is significantly associated with nodal metastasis

- Stage II CRC (pT3/4 pN0 M0)
  - Heterogeneous group; risk stratification needed ➔ high-grade tumor budding as a “high risk feature”
    - High-grade tumor budding associated with poor overall and disease-free survival in resected patients with stage II disease
    - Tumor budding associated with other aggressive clinicopathologic features (i.e., LVI, higher tumor grade, infiltrative tumor margin)

Tumor budding: What to do with the information??

- Malignant polyps
  - Tumor budding as a predictor of lymph node metastasis
  - Tx/management: Surgical resection

- Stage II CRC
  - Tumor budding as an adverse prognostic factor
  - Tx/management: Risk-adapted follow-up and adjuvant therapy

- Pre-operative biopsies of CRC
  - Tumor budding as an adverse prognostic factor and predictor of lymph node and distant metastasis
  - Tx/management: Neo-adjuvant therapy and risk-adapted surgery

So HOW do you count tumor buds??

Multitude of methods...
Meta analyses

- Despite multitude of methods, tumor budding in CRC is strongly predictive of:
  - Lymph node metastases
  - Recurrence
  - Cancer-related death at 5 years

### 2016 Consensus Statements

- **DEFINITION of Tumor Budding:**
  - Single tumor cells or clusters of up to 4 cells at the invasive margin

- **Tumor Budding ≠ Tumor Grade**

- **Tumor Budding should be counted on H&E (not cytokeratin), using hotspot method**
  - Scan the entire invasive front in all tumor sections and choose a "hotspot"
  - Count # tumor buds in a 20x field
  - Apply appropriate correction factor for your microscope to get count in 0.785 mm² (Ueno method)
  - Provide tumor budding score (low/intermediate/high)
**Method: Ueno et al.**

- **Tumor bud definition:** <5 cancer cells, observed in the invasive frontal region.
- **Method:** Clusters were counted under the 20x objective lens in a field where budding was observed most intensively.
- **Grading tumor budding:**
  - G1: <5
  - G2: 5 to 9
  - G3: ≥10

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**Why use the Ueno method?**

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**Method: Consensus 2016**

**THE PROPOSED METHOD**

1. Select the best H&E slide for budding
2. Scan in 10 fields at low magnification the hotspot at the invasive front
3. Pay attention at the conversion at your microscope
4. Count the buds in a field area of 0.785mm²
5. Report budding

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**HOW TO REPORT TUMOR BUDDING**

- **Budding 1 (x buds / hotspot, 0.785mm²; low)**
- **Budding 2 (x buds / hotspot, 0.785mm²; intermediate)**
- **Budding 3 (x buds / hotspot, 0.785mm²; high)**

- Low: 0-4 buds
- Intermediate: 5-9 buds
- High: ≥10 buds

Proposed abbreviation for TNM: Bd1, Bd2, Bd3
The UCSF Experiment (Round 1)

- 10 total faculty who sign out GI cases (primary or secondary area)
- Tumor budding previously discussed at departmental subspecialty meetings
- Brief Powerpoint with background and recent consensus methodology
- Whole slide image scanning (Aperio) utilized
  - 10 cases of colorectal carcinoma selected (random)
  - Two circled areas corresponding to 20x field diameter on UCSF microscopes

For UCSF Microscopes (BX40/50):

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For tumor budding scores >12, multiply by 0.8

So we actually tried it...
The UCSF Experiment (Round 1)

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Comments and Points for Discussion

- “How close...??” – distance between clusters, distance of cluster to larger gland
- “Where to count?? Does it have to exactly be at the “leading edge” only or can it be a little more superficial??” – peritumoral versus intratumoral budding
- “What to do with very poorly differentiated tumors??”
- “Glandular fragmentation vs. true budding”
- “Retraction may make some clusters appear like separate clusters? Crushed cells? Degenerating cells?”
- “I probably undercounted as I tried to ignore fibroblasts but some of them may have been tumor cells.”

Challenges

- **Technical**
  - H&E versus Cytokeratin
    - Cytokeratin staining results in tumor bud counts that are 3-4x counts obtained on H&E
- **Interpretive**
  - Gland fragmentation
  - Inflammation obscuring tumor buds
  - Tumor bud versus stromal cells

Infiltrative border but no tumor budding
Tumor budding in a malignant polyp (arrows)

Blurring of tumor-stroma interface

Blurring of tumor-stroma interface

Higher magnification reveals tumor budding

Challenging scenarios

Peritumoral inflammatory infiltrate

Tumor vs. stromal cells
Challenging scenarios

Neoplastic glandular fragmentation (arrow, not tumor bud)

Recommendations by Mitrovic et al.

- Report tumor budding in all malignant polyps and CRC resection specimens
- Ueno methodology
  - “We report tumor budding as present if ≥10 groups of <5 cells are counted in a 20x objective field (i.e., Ueno’s so-called ‘high-grade budding’).”
- In “borderline” cases ➔ Cytokeratin
  - “If this confirm the impression of additional tumor cells, bringing the count to ≥10 buds, we report as positive for tumor budding.”
  - “However, we caution against the routine use of cytokeratin stains in cases where bud counts on H&E do not approach 10/20x objective. In our experience, counts by cytokeratin immunohistochemistry are substantially higher than those on H&E and the limited data suggest that much higher cutoffs are needed to reach prognostic significance.”

Lessons Learned / Future Directions

- Easy concept, difficult to put into everyday practice
  - “Tutorial” may be helpful
- What to do when you really can’t count??
  - Cytokeratin ➔ Go back to H&E and count... (UCSF Round 2?)
- Consensus = “Correct” method??
  - Additional studies necessary...

Method: Rieger et al. (2017)

PTB (peritumoral budding)

ITB (intratumoral budding)

OTB: Hotspot with most budding (PTB_Hotspot or ITB_Hotspot)

OTB_Hotspot: 10 HPF with most budding out of all acquired 20 HPF

“Special variants” ➔ NOT tumor buds

- **Micropapillary**
  - Per WHO 2010, “small clusters of tumor cells within stromal spaces mimicking vascular channels”
  - Same as poorly differentiated cell clusters (PDCs)??

- **Mucinous**
  - Cell clusters lie in mucin pools and are not surrounded by tumor stroma ➔ Do NOT qualify as bona fide tumor buds
  - Excluded from assessment of tumor budding

- **MSI-H**
  - Tumor budding virtually absent

**Micropapillary variant**


**Mucinous variant**

**“Special variants” ➔ High grade buds**

- **Signet ring cell**
  - Suggested to classify as *high-grade tumor budding by definition* (Prall F. Histopathology. 2007;50:151-62.)
Take-home messages

• Tumor budding is an emerging important independent prognostic factor in colorectal carcinoma

• Methodology is undergoing refinement
  – H&E vs. Cytokeratin
  – What to report

• Watch for future updates in reporting

The UCSF Experiment (Round 1)

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Infiltrative border but no budding

Resection specimen – blurring of tumor-stroma interface

Tumor budding in a malignant polyp (arrows)

Higher mag of (c) – tumor budding seen