Pilot Study on Interval Colon Cancer: Missed Colon Cancer or Fast Growth from a Serrated Polyp?

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Pilot Study on Interval Colon Cancer: Missed colon cancer or fast growth from a sessile serrated polyp?

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Introduction

Colorectal cancer is the third most common cancer diagnosed amongst men and women, as well as the second leading cause of cancer-related deaths in the United States[1]. Early detection and treatment are associated with a better survival outcome. The American Cancer Society recommends regular screening procedures, such as a colonoscopy, at age of 50 for both men and women[2].

Three distinct molecular pathways have been described in colorectal cancer[3]: The APC/Beta-catenin pathway, the microsatellite instability pathway, and the serrated neoplasia pathway (SNP). Sessile serrated polyps (SSP) (Figure 1) represent a precursor lesion in 10% of all colorectal cancers and are often associated with BRAF mutations (over 70% of SSP)[3]. These lesions occur commonly in the proximal right side of the colon. SSP polyps are flat lesions that can be difficult to visualize on colonoscopy screenings. It is hypothesized that early interval colon cancer after a negative colonoscopy may be a result of missed SSP, which may have a more rapid progression to neoplasia. BRAF positive colorectal carcinomas have been associated with old age and are frequently of a higher grade[4]. Microsatellite instability and CpG (5'-cytosine-phosphodiester bond-guanine-3') island methylation are also associated with the SNP and specifically with the BRAF mutation in colorectal carcinoma[5].

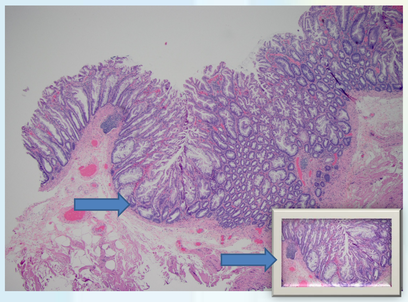

Figure 1. *Sessile serrated polyp*. Hematoxylin and Eosin stain, original magnification x10. **Insert:** Sessile serrated polyp with saw-tooth branching crypts and flask-like expansion at the base, original magnification x 40.

Design and Aim of the Study

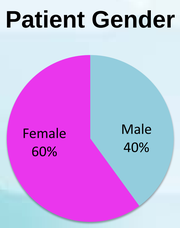

Figure 2a. *Patient Gender.* Ten males (40%) and fifteen females (60%).

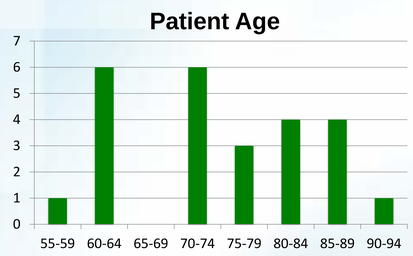

Figure 2b. *Patient Age.*

The aim of this study is to determine if a BRAF mutation is associated with interval colorectal cancer in patients who have had a negative colonoscopy within the preceding 5 years, and therefore may have been associated with an SSP precursor lesion and/or the SNP pathway. A negative colonoscopy is defined as a colonoscopy without evidence of any malignancy, clinically and microscopically. The interval time of the study is between 7 May 2009 and 21 November 2014. 268 patients were identified with invasive colorectal carcinoma during that time period. Of these, 25 patients (15 females and 10 male) (Figure 2a) fulfilled the inclusion criteria. Patient's age ranges were between 56-94 (Figure 2b).

Materials and Methods

Microscopic localization of tumor

The diagnosis of invasive carcinoma was confirmed by histologic criteria (Figure 3a). The H and E slides were reviewed and areas representing tumor/invasive adenocarcinoma were marked for paraffin block macro-dissection. Paraffin blocks with designated tumors were cut at 5 sections, 10 microns each.

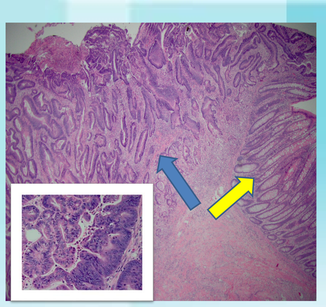

Figure 3a. *Normal colonic mucosa (yellow arrow) transitioning to an invasive adenocarcinoma (blue arrow).* Hematoxylin and Eosin stain, original magnification x 10. **Insert:** Invasive adenocarcinoma, original magnification x 40.

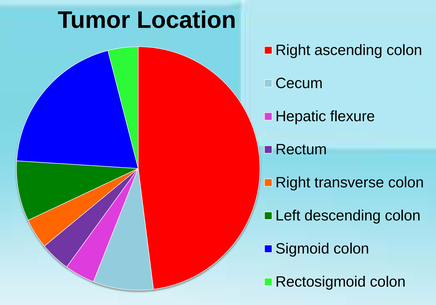

Figure 3b. *Tumor Location*. Location of the invasive adenocarcinoma within the patients in the study.

DNA extraction:

The cut sections were submitted for DNA extraction (using the Qiagen protocol) and subsequent PCR amplification for BRAF detection.

BRAF detection:

The Entrogen BRAF Mutation Analysis Kit was used. It is intended for the detection of the following BRAF mutations in human genomic DNA at codon 600:

- GTG → GAG (V600E)
- GTG → AAG (V600K)
- GTG → GAT (V600D)

- The BRAF mutation analysis real-time assay is based on mutation-specific PCR. Mutation-specific PCR uses primers that are 100% complementary to mutant variants of the gene. The assay also amplifies an internal control gene in order to ensure that sufficient amount of DNA is available for amplification.
- The detection of the amplification product is done by using fluorescent hydrolysis probes. Each probe contains a fluorophore (FAM™ for the mutant variant of the BRAF gene or VIC® for the internal control gene).
- The probes are complementary to the regions of interest and hybridize to the template DNA. During the amplification process, DNA polymerase cleaves off the fluorophore and the quencher from the probe. Upon separation from the quencher, the fluorescence signal increases dramatically, which is seen by the instrument detectors.
- The PCR detection instrument used in the study is the Roche LightCycler 480.
- Sensitivity of the assay: the assay is able to detect 1% mutation in a background of wild-type DNA .

Results

Ten out of twenty-five (40%) of the patients with invasive colorectal carcinoma had a BRAF V600E mutation (Figure 4a). 80% of these patients were female (Figure 4b), and 90% of the patients were over the age of 70 (Figure 4c). 70% of the tumors with the BRAF mutation were located in the right ascending colon (Figure 4d).

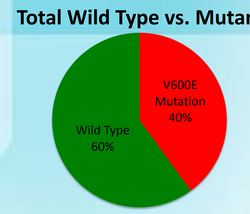

Figure 4a. *Total Wild Type vs. Mutant.* Percentage of patients with a mutation and without a mutation.

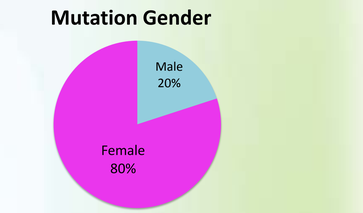

Figure 4b. *Mutation Gender.* Gender of patients with a BRAF mutation.

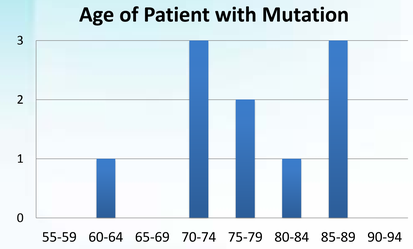

Figure 4c. *Age of patients with a BRAF mutation.*

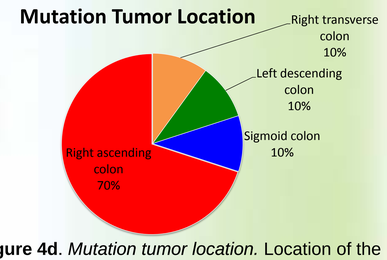

Figure 4d. *Mutation tumor location.* Location of the invasive adenocarcinoma within patients that have a BRAF mutation.

Conclusion and Future Direction

The data from our institution is in accord with previous publications and demonstrates the propensity of interval colorectal cancer to occur within the right side of the colon[6]. It also highlights the association of these cancers with BRAF mutation, the sessile neoplastic pathway, older age, and female gender[7]. The study also supports the hypothesis that interval colorectal carcinoma following a negative colonoscopy might indicate a missed SSP as the precursor lesion in the right side of the colon in a significant subset of the cases. Future direction is aimed at improving the screening process for colorectal carcinoma. Additional testing includes the possibility of serologic screening and better preparation/visualization of the right side colon, which may facilitate early detection of precursor lesions. Enhanced clinical experience with colonoscopies and awareness of the subtle nature of these precursor lesions may also establish a better preventive strategy in a subset of the patients.

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8. Microscopic images produced by Dr. Shereen Gheith and Dr. Jillian Grau (Figures 1 and 3a). 21 Jul 2016.