Most colorectal cancers involve gene mutations, and genes upregulated include cyclin D, used as a diagnostic marker for colon carcinoma. BCL-6 is a transcriptional regulator important in lymphocyte survival. Microarrays show that, in colorectal cancer, BCL-6 is 7-fold upregulated compared to controls. Could BCL-6 be used as a diagnostic marker? With IRB approval, I obtained human colon tissue taken in surgical resection, diagnosed as “moderately differentiated adenocarcinoma of colon”. My question: what percentage of these diagnosed cases would stain positive for cyclin D (control) and for BCL-6?

Sections with the diagnosis of moderately differentiated adenocarcinoma of colon were stained. Of the 100% surgically diagnosed cases, both positive, or both negative. Of the 100% surgically diagnosed cases, BCL-6 was positive in 16% and cyclin D positive in 29%. BCL-6 was significantly related to cases where mucin was present, significant “inflammation” was noted on the report, p<0.01. This study attempted validation of a potential colon cancer biomarker from surgical specimens. A need exists to identify tumor-positive tissue as well as to predict treatment responsiveness or explain mechanisms.

METHODS AND MATERIALS

- **CLEARANCE** was requested from the UMKC IRB; Paraffin tissue blocks were obtained from pathology by the resident and slides were number coded; surgical pathology notes were coded to the slides and no patient contact was made.

- **TISSUES:** Sixty two blocks with the diagnosis of moderately differentiated adenocarcinoma of colon were sectioned and given a code letter, A – JJJ. Copies of the surgical pathology reports were obtained by the resident, also coded to match the sections.

- **STAINING:** Primary monoclonal antibodies vs Cyclin D and BCL-6, anti mouse HRP secondary and DAB substrate were utilized for immunohistochemistry. Sections were heated for antigen retrieval, stained overnight with the primary and 1 hr with secondary antibodies. After substrate, slides were cover-slipped for permanent storage.

- **PHOTOGRAPHY:** Slides were photographed at 400x and 1000x.

- **EVALUATION** Stained sections were classified as “stained” or “no stain” by a pathology resident, medical student, a researcher, and two pathology faculty.

- **DATA SUMMARY:** Data collected: number of positive nodules; presence or absence of: mucin in tumor; inflammation; polyp origin. Numbers were keyed into Statistica (Statsoft, Tulsa OK), and summaries were made.

**RESULTS**

**RESULTS I**

- **INTRODUCTION**
  - The majority of colorectal cancers involve gene mutations to the important Wnt gene pathway
  - Important genes upregulated by Wnt signaling include those for Cyclin-D, used clinically as a diagnostic marker
  - BCL-6, a transcriptional regulator that is important in B lymphocyte survival and differentiation
    - Recent microarray research evaluated colorectal cancer pathways
    - BCL-6 marker was 7-fold upregulated, CA vs control

**RESULTS II**

- **SUMMARY AND CONCLUSIONS**
  - BCL6 is present in unselected colon adenocarcinoma cases from TMC but is only 16% positive in these surgically diagnosed cases
  - BCL6 was significantly related to cases where mucin was stained or the tumor was called “mucinous”
  - BCL6 staining correlated with slightly higher number of nodes positive compared to Cyclin-D staining or no stain
  - BCL6 was positive in only 5 of 31 complete cases compared to 9 of 31 Cyclin D positive
  - In cases positive for both markers, there was significantly more “inflammation” cited on the surg. pathology report

- **LIMITATIONS**
  - Small numbers of patients available for study at TMC
  - Colorectal cancers may involve other genes (untested)

- **REFERENCES**