

Identification of tumor associated antigens for the development of a diagnostic test for colon cancer

Key mentors: N. Disis, H. Lu

Overall goals of the project (BIG PICTURE):

The overall goal of the project is to develop a panel of autoantibodies that are effective in discriminating colon cancer patients from controls. Previous work by the group has identified a 4 antigen panel that performed better than standard non-invasive assays in this regard (attached). Work performed in this project will expand on the panel for the purpose of increasing both the sensitivity and specificity of the approach. Experiments will focus on the serologic screening of the immune response via SEREX. The SEREX technique is based on using serum to screen cDNA libraries derived from specific tumors. Two commercial libraries will be used; a colon cancer and testis libraries. Initially, sera from volunteer donors and early stage colon cancer patients will be evaluated. A second component of the project will be to assess for similar Ab responses in a transgenic murine model of colon cancer. This model may also be exploited for therapeutic purposes. Antigens identified above could be used for vaccine development. The final goals of the project will be to develop protein vaccine candidates based on their biologic function in colon cancer oncogenesis. These proteins will then be prospectively screened for immunogenicity in humans and their therapeutic potential as vaccine candidates in animal models.

Specific aims:

- (1) To identify tumor antigens which generate autoantibodies in patients with early stage colon cancers as compared to controls that increase Sn and Sp of the current diagnostic panel.
- (2) To determine the early antigenic repertoire in a transgenic model of colon cancer and assess whether those autoantibodies predict cancer development.
- (3) To identify candidate overexpressed proteins involved in colon cancer progression and determine whether those proteins are immunogenic in patients.

SUMMARY

YEAR 1-2

1. To identify tumor antigens which generate autoantibodies in patients with early stage colon cancers as compared to controls that increase Sn and Sp of the current diagnostic panel.

- Begin screening colon and testis libraries with pool of 10 donors and 10 early colon
- Identify reactive clones
- Screen individual sera using crude lysate ELISA
- Develop his-tag ELISA for promising candidates'

RESOURCE PEOPLE

Hailing Lu

Mei Wu (SEREX and ELISA)

2. To determine the early antigenic repertoire in a transgenic model of colon cancer and assess whether those autoantibodies predict cancer development (e.g. Lu H. et al Cancer Research, 2006).

- Obtain and breed animals
- Establish serum library
- Develop tumor cell line from syngeneic tumor
- Create cDNA library

- Serologic screening

RESOURCE PEOPLE:

Ekram Gad (Animal breeding and sample collection)

Ekram Gad (creation of tumor cell lines)

Hailing Lu (Library construction)

3. To identify candidate overexpressed proteins involved in colon cancer progression and determine whether those proteins are immunogenic in patients (e.g. Park K. et al Cancer Research, 2008).

- Literature search and preparation of lab meeting
- Selection of candidates
- ELISA screening of PBMC
- in silico epitope prediction
- Peptide construction
- Screening human PBMC for reactivity by ELISPOT

RESOURCE PEOPLE

Vy Lai, Nora Disis (in silico screening)

Mei Wu (ELISA development)

Yushe Dang/Meredith Slota (ELISPOT screening)

FIRST 18 MONTH WORK PLAN

*** schedule is made for 12 months*

1. To identify tumor antigens which generate autoantibodies in patients with early stage colon cancers as compared to controls that increase Sn and Sp of the current diagnostic panel

Months 1-2

- Identify sera and create pools of experimental and controls
- Train with Dr. Mei Wu on SEREX screening and sera preparation
- Screen human testes library
- Order colon cancer library and screen with SEREX

Months 3-4

- Continue primary screening of testis and colon cancer library
- Secondary screening to rule out non-specific autoantibodies
- Purifying positive clones and identifying the antigenic protein by sequencing after SCE

Months 5-6

- Screening of libraries and identification of candidates
- Purification of candidate clones
- Establishing crude lysate ELISA to evaluate individual response

Months 7-8

- Screening of libraries and identification of candidates
- Purification of candidate clones
- Begin screening of individual response with crude lysate ELISA

Months 9-10

- Screening of libraries and identification of candidates
- Purification of candidate clones
- Begin screening of individual response with crude lysate ELISA
- Identify candidates for validation
- His tag development of top candidates

Months 11-12

- Begin validation studies of candidates in archived sera panels
- Evaluate additive effect

FUNDING PLAN:

R01 Application Hailing Lu 10/09-Liz with salary support as Co-I

Develop concepts using data to support new application (adenomatous polyp sera or UC with colon vs. UC without colon) for a GIM application-R01 vs. R21-02/10-Liz as PI

2. To determine the early antigenic repertoire in a transgenic model of colon cancer and assess whether those autoantibodies predict cancer development

Months 1-2

- Obtain mice

Months 3-4

- Breed mice

Months 5-6

- Breed mice
- Collect sera

Months 7-8

- Breed mice
- Collect sera
- Establish tumor cell lines

Months 9-10

- Breed sera
- Collect sera
- Establish tumor cell lines
- Begin screening Ag repertoire (student helper)

Months 11-12

- Screening and Ag identification
- Plan therapeutic experiments with any of the candidates identified

THIS AIM CAN BE DELAYED SOMEWHAT IF OTHER AIMS ARE PRODUCING SIGNIFICANT DATA

3. *To identify candidate overexpressed proteins involved in colon cancer progression and determine whether those proteins are immunogenic in patients*

Months 1-2

- Literature review
- Candidate identification
- PBMC collection plan in colon cancer patients

Months 3-4

- Lab meeting discussion and justification of candidates
- Begin in silico analyses of top candidates
- ELISA development for serologic screening of candidates (indirect)
- Collect PBMC in colon cancer

Months 5-6

- in silico analyses of top candidates
- ELISA for serologic screening of candidates (indirect)
- Initial ELISPOTS

Months 7-8

- ELISA for serologic screening of candidates (indirect)
- ELISPOT

Months 9-10

- Validation of T cell responses of positive candidates via generation of T cell libraries

Months 11-12

- Begin to plan application of positive candidates (ELISA) to diagnostic assay
- Assess homology with murine model and begin to characterize murine tumors for expression

FUNDING PLAN: Career development award at the end of 12 months based on this data (ASCO/AACR/Other)

ADDITIONAL:

- Recruit cancer patients for leukapheresis (clinic, advocacy groups, etc)
- Identify all national and academic specific serum banks of characterized colon cancer sera