Research Proposal On

IDENTIFICATION OF BRONCHOGENIC CANCER MARKER(S) THROUGH PROTEOMIC ANALYSIS OF BRONCHIAL ALVEOLAR LAVAGES AND SERA

Submitted for funding to

Department of Science and Technology, New Delhi

BY

Principal investigator
Dr. Raies Ahmad
Associate Professor

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University of Kashmir
Srinagar, Kashmir
101. Title of the project:

“Identification of Bronchogenic Cancer Marker(s) Through Proteomic Analysis of Bronchial Alveolar Lavages and Sera”
102. Broad subject: Life sciences
103. Sub area: Biophysics, Biochemistry and Molecular Biology.
104. Duration in months: 36 months (3 years)
105. Total cost: Rs 62,03,600
106. FE Component: 1,24,295 USD
107. Project Category: Basic Research
111. Principal Inv.: Raies Ahmad
112. Designation: Associate Professor
113. Department: Biotechnology
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    Fax: 0194-2428723
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127. Department: Medicine

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Project Title:
“Identification of Bronchogenic Cancer Marker(s) Through Proteomic Analysis of Bronchial Alveolar Lavages and Sera”

Registration no._________________________________________ (to be filled by DST)

Principle Investigator: Dr. Raies Ahmad.
Institution: Department of Biotechnology, University of Kashmir.

191. Project summary:

Lung cancer continues to be a leading cause of cancer mortality in the developing as well as the developed world. Patients suffering from this cancer have a 10-15% overall survival despite advances in chemotherapy, radiation therapy and surgery. This is mainly because the diagnosis is made at a very late stage of the disease. 80% lung cancer is attributed to smoking and the relative risk of lung cancer is 20 times greater in smokers [1]. However, for several years now more and more cases of lung cancer are being detected in non-smokers which suggest that there might be a genetic factor contributing to susceptibility to this cancer [2-6]. Metabolites derived from cigarette smoke can form adducts with DNA and contribute to carcinogenesis. Such adducts have been reported in smokers and lung cancer patients, and lead to mutations similar to those found in subjects with p53 mutant gene or mutant ras oncogene. A number of oncogenes have been shown to be upregulated or modified in lung cancer patients [7,8]. These include c-myc, raf, ras, EGFR and many others [9-12]. Similarly chromosomal alterations have been reported in lung cancers and immortalized bronchial epithelial cell lines [13]. Furthermore, alterations in tumor suppressor genes including p53 and retinoblastoma gene (Rb) have also been demonstrated. EGFR has been of particular interest because small molecules that inhibit signaling from this receptor have been investigated as therapeutic agents that might be particularly useful for non-small cell lung cancer (NSLCC) patients. However, in spite of technological advancements, the molecular signatures of the events that lead to bronchial carcinogenesis as well as biomarkers that could lead to early and specific diagnosis of the disease remain unidentified. For any anti-cancer therapeutic strategy to be effective, early laboratory diagnosis is of paramount importance. The present proposal will be
undertaken to carry out a proteomic analysis of the bronchial lavages and sera from bronchogenic cancer patients and to investigate anti-cancer specific/associated antibodies in these patients.
192. Key words: Lung cancer, VGFR, BALF, Early Lung cancer markers.
200. Technical details:

210. Introduction

211. Origin of the proposal

Lung cancer is the most common cause of cancer-related death in men and the second most common in women. It is responsible for over 1 million deaths worldwide annually [2]. This cancer is produced due to uncontrolled growth of the bronchial epithelial tissue in the lung, the most common cause being exposure to tobacco smoke. However, in recent years the incidence of lung cancer in non-smokers has shown an increasing trend [2,3]. Genetic factors, air pollution, asbestos, Radon gas, viral infection and ionizing radiation are believed to contribute to the occurrence of this cancer in non-smokers. There are two main types of lung cancer – small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC is the most common lung cancer and is strongly associated with smoking. This type of cancer is initially more sensitive to chemotherapy but ultimately carries a worse prognosis and is often metastatic at presentation [14]. NSCLC can be further classified into three main types – squamous cell lung carcinoma, adenocarcinoma and large cell lung carcinoma accounting for 29%, 32% and 9% of lung cancers respectively [2,4,15]. The diagnosis of lung cancer is made by performing a chest X-ray. Confirmation and identification of the type is made by bronchoscopy or CT-guided biopsy. In majority of the cases, this diagnosis is made at a time when the cancer has already reached an advanced stage. Treatment of this cancer depends on cancer’s specific cell type, the stage and patient’s status, and the modalities include surgery, chemotherapy and radiation therapy. Chemotherapy includes cisplatin or carboplatin in combination with gemcitabine, paclitaxel, docetaxel and etoposide [16-19]. In recent years, targeted therapies have been developed for the treatment of advanced lung cancer. These include Gefitinib – EGFR tyrosine kinase inhibitor, Erlotinib – another tyrosine kinase inhibitor, angiogenesis inhibitor bevacizumab (given in combination with cabroplatin and palcitaxel; [20,21]. However, even with limited stage lung cancer, the 5 year survival rate after any kind of therapy is just about 20%. Thus there is an urgent need to identify a marker or markers that would enable early diagnosis, and to develop more effective and specific therapeutic regimen(s) for the treatment of this cancer. It is with this objective in mind that the present study proposes to carry out a proteomic analysis of bronchial
lavages and sera obtained from lung cancer patients, and analyse presence of antibodies that might be generated in patients in the course of oncogenesis.

212. Definition of the problem

Bronchogenic cancer is one of the most lethal types of cancer. More than 50% patients have advanced stage incurable disease at the time of diagnosis. Moreover, the majority of those with potentially curable NSCLC at diagnosis will relapse with incurable disease within a few years. SCLC which is in general initially responsive to chemotherapy metastasizes very fast. Even the most aggressive chemotherapy currently in use offers only about 20-25% chance of tumor response and an improvement in survival by an average of 2-4 months. The molecular events governing oncogenesis include upregulation or downregulation of molecules that might be directly or indirectly involved in cell proliferation and related events, or may be mutations leading to for example increased kinase activity of a membrane receptor such as EGFR. Cancer cells might also secrete molecules that might be involved in regulating cell growth, metastasis or downregulating host immunity.

In recent years, small molecule inhibitors of EGFR which have been remarkably successful with breast cancer have been introduced into clinical trials for the treatment of NSCLC. These molecules target the tyrosine kinase domain of EGFR and inhibit intracellular signaling. Engagement of the receptor brings about activation of the cytoplasmic domain initiating a cascade of intracellular signaling events that culminate in cell proliferation. The transforming ability of aberrant EGFR signaling has been demonstrated in multiple cell types including NSCLC [10,22]. Recent studies suggest that many lung cancer patients have EGFR with mutations in the kinase domain. Therefore, EGFR directed therapy might be effective in a small number of lung cancer patients. This approach would of course require prior genotyping of this receptor. However, it is clear that while designer chemotherapeutic agents based on genotyping of molecules such as EGFR might be a proposition worth pursuing, on a long term basis and particularly in the developing world this might not be a viable proposition. More importantly, any kind of therapy would be more effective when the cancer burden is low and cancer cells have not metastasized. Therefore there is a need to identify a marker or markers that would enable early diagnosis.
**213. Objectives:**

1. To carry out a proteomic analysis of bronchial lavages and sera samples obtained from bronchogenic cancer patients.
2. To analyze proteins secreted by model human lung cancer lines such as A549 and CHAGO.
3. To investigate usefulness of a molecule(s) identified by proteomic analysis in the laboratory diagnosis of lung cancer.
4. To analyze antibodies against cancer-specific/ associated antigens in the sera of lung cancer patients.

**220. Review of status of Research and Development in the subject**

**221. International status**

The understanding of oncogenesis, development of specific methods for early diagnosis of cancers and development of specific therapeutic agents are major thrust areas all over the world. Lung cancer ranks amongst the leading causes of cancer deaths world wide with over 1 million deaths every year. Most cases of lung cancer are due to tobacco smoking. However, recent studies suggest that 15% of lung cancer in men and about 53% in women are not attributable to smoking and collectively, lung cancer in non-smokers is the seventh leading cause of cancer death in the world. The incidence in non-smokers is highest amongst Asian women [3,23-29]. The reasons for this are not well understood although hormonal, genetic and viral factors have been suggested [30-33]. At the molecular level, interestingly, the mutations found commonly in molecules such as ras, p53 and EGFR amongst lung cancers from smokers are different from those reported amongst lung cancers from non-smokers. The identification of causes of lung cancer amongst non-smokers therefore becomes very important. Recently, tyrosine kinase inhibitors targeting EGFR have shown promise in the treatment of NSCLC. Sustained and quite dramatic responses to these drugs amongst a small number of NSCLC patients have been attributed to mutations in EGFR [34-38]. These preliminary but encouraging results have led to more investigations on the use of these inhibitors in the treatment of NSCLC. It is important to point out that this treatment has been shown to work on a small number of patients and would require prior EGFR genotyping of the patients. Several studies have reported molecules that
might be upregulated in the BALF and SERA of cancer patients and can act as biomarkers like fibronectin, melanoma antigen-A, Neutral endopeptidase, Carcinoembrogenic antigen etc [40-44]. Many studies have also demonstrated the presence of antibodies against cancer related antigens in patients [45]. However, to the best of our knowledge no extensive analysis on the proteome of bronchial alveolar lavages or sera of lung cancer patients has been reported so far.

222. National status

In India most of the work has been done clinically in different hospitals. Infact scientists are more focused on the patients having history of smoking and they propose that lung cancer in most parts of India is due to cigarette smoking. People have also identified genetic mutations in some populations and believe genetic disorder is one of the factors for lung cancer. In Kashmir such type of study has not been done so far and we also believe the major cause may be cigarette smoking as the region comes under pollution free zone. Studies have shown that in India also lung cancer is a leading cause of cancer death but there is no study as such where people have identified different markers in Indian population.

REFERENCES:


223. Importance of the proposed project in the context of current status

For any anti-cancer therapeutic strategy to be effective, early laboratory diagnosis is of paramount importance. Among the important tools critical to detection, diagnosis, treatment, monitoring, and prognosis are biomarkers or tumor markers. The utility of a biomarker lies in its ability to provide an early indication of the disease, to monitor disease progression, to provide ease of detection, and to provide a factor measurable across populations. Detection of cancerous state at an earlier stage increases the survival chances of the patients. Several approaches have been evaluated for the early detection of lung cancer, but screening strategies have failed to decrease disease-specific mortality. Exfoliated cells shed from the lower respiratory tract can be detected in (induced) sputum or in BAL samples and can be helpful for the detection of central tumors of the larger bronchi. Their presence in the bloodstream as well, even though in small amounts, suggests that their assay might represent a novel approach in the assessment of lung diseases with still elusive pathogenesis, prognosis, diagnosis and therapeutic interventions.

Proteomic technologies allow identification of the protein changes caused by the disease process in a relatively accurate manner. The inherent advantage afforded to proteomics is that the identified protein is itself the biological endpoint. At the protein level, distinct changes occur during the transformation of a healthy cell into a neoplastic cell, including altered expression, differential protein modification, changes in specific activity, and aberrant localization, all of which may affect cellular function. Identifying and understanding these changes is the underlying theme in cancer proteomics.

The present study proposes to carry out a proteomic analysis of bronchial lavages and sera obtained from lung cancer patients, and analyze presence of antibodies that might be generated in patients in the course of oncogenesis with an aim to identify a specific biomarker for early detection of lung cancer.

224. Review of expertise available with proposed investigating group/institution in the subject of the project.

Protein Expression Studies:
Our group is involved in expression studies of VEGFR-2 from BALF of lung cancer patients from different parts of this region. We have been engaged in collecting patient samples from Medical Institutes and establishing the data for patients having different history. So far we have been able to establish a correlation of VEGFR-2 expression with poor and moderately differentiated SCC as well as adenocarcinoma, constituting a major chunk of NSCLC.

Cell culture studies:
A number of animal cell lines (malignant as well as non-malignant) are being maintained presently by our group for various molecular and immunological studies. Their proteome analysis and 2D electrophoresis standardization is currently under progress.

225. Patent details (domestic and international):
Not Applicable

230. Work plan

231. Methodology

Two dimensional gel electrophoresis
Sera samples and bronchiolar alveolar lavages (BAL) from clinically confirmed bronchogenic cancer patients will be collected in accordance with the guidelines provided by the SKIMS Institutional Ethics Committee. Samples will be centrifuged at 12,000 x g and stored at –70°C till the time of analysis. Samples will be subjected to 2D gel electrophoresis as described by Wu et al. (39). 2D analysis will also be carried out after removing albumin from serum (and may be lavages as well) using anti-albumin antibody affinity matrix. Controls will consist of patients whose lavages/ Sera have been taken for suspected non-cancer ailments such as infection. Comparison between cancer and non-cancer samples will be made by subjecting the 2D electrograms to software analysis provided with the 2D system. The identity of molecules (spots in the 2D electrogram) of interest will be established by mass spectrometric analysis which will
be carried out through a commercial source (for example TCGI, New Delhi). The sequences will be blasted against the NCBI protein sequence bank.

**Analysis of secretory proteins from lung cancer cell lines A549 and CHAGO**

A549 and CHAGO, model human lung cancer lines, representing lung adenocarcinoma and squamous cell lung carcinoma respectively will be obtained from American Type Tissue Culture and grown according to the instructions provided by the supplier. Supernatants collected from these cell lines and also from a Cervical Epithelial cell line, HeLa, after growing cells under serum-free conditions will be concentrated and analyzed by 2D gel electrophoresis. The identity of the spots will be determined by mass spectrometric analysis.

**Generation of antibodies and Western blotting**

Antibodies against molecules identified through proteomic analysis, if not available commercially, will be generated through a commercial source by injected one or more peptides (chosen from the amino acid sequence of the protein of interest) linked to an appropriate carrier (such as KLH). These antibodies will be employed to carry out immunoblotting with sera and bronchial lavages. Further studies will be carried out depending upon the results obtained through this analysis.

**Analysis of antibodies in patients**

Sera and bronchial lavages from lung cancer patients and non-cancer controls will be analyzed for the presence of antibodies against using lung cancer cells (A549 lung cancer cell line) or molecules secreted by this cell line as the source of antigen. This analysis will be carried out ELISA and immunoblotting using standard techniques.
233. Time schedule of activities giving milestones (also append to bar diagram and mark it as Section 410)

Ist year (0-12 months):
Ordering of equipment & cell lines, appointment of project assistants and collection of patient samples. Standardization of proteomics

IIInd year (12-24 months):
2D gel analysis of samples from BALF and sera and their molecular characterization. Collection and analysis of secretory proteins from cancer cell lines. Analysis of antibodies in patients’ sera and lavages.

IIIrd year (24-36 monthhs):
Characterization of proteins obtained through proteomics and generation of antibodies, and probing of BALF and sera with these antibodies.

234. Suggested plan of action for utilization of research outcome expected from the project.

This study should reveal identity of molecules that could be employed as markers for specific and possibly early detection of lung cancer.
### 300. BUDGET ESTIMATES: SUMMARY

<table>
<thead>
<tr>
<th>S.no.</th>
<th>ITEM</th>
<th>1&lt;sup&gt;ST&lt;/sup&gt; YEAR</th>
<th>2&lt;sup&gt;ND&lt;/sup&gt; YEAR</th>
<th>3&lt;sup&gt;RD&lt;/sup&gt; YEAR</th>
<th>TOTAL</th>
</tr>
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<td></td>
<td></td>
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<td>1.</td>
<td>Salaries/Wages</td>
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<td>3,31,200.00</td>
<td>3,31,200.00</td>
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<td>12,00,000.00</td>
<td>31,00,000.00</td>
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<tr>
<td>3.</td>
<td>Travel</td>
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<td>30,000.00</td>
<td>30,000.00</td>
<td>90,000.00</td>
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<tr>
<td>4.</td>
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<td>40,000.00</td>
<td>40,000.00</td>
<td>1,20,000.00</td>
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<tr>
<td>B.</td>
<td>Equipment</td>
<td>19,00,000.00</td>
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<td>-</td>
<td>19,00,000.00</td>
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<td></td>
<td>GRAND TOTAL (A+B)</td>
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<td>15,01,200.00</td>
<td>16,01,200.00</td>
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<td>TOTAL FEC*</td>
<td>62,135.80USD</td>
<td>30,078.10USD</td>
<td>32,081.70USD</td>
<td>1,24,295.00USD</td>
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</tbody>
</table>

### 310. BUDGET FOR SALARIES/WAGES

<table>
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<tr>
<th>Designation and no. of persons</th>
<th>Monthly Emoluments</th>
<th>BUDGET</th>
<th>(In Rupees)</th>
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</thead>
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<tr>
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<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Year</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Year</td>
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<tr>
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<td>12000+1800HRA</td>
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<td>(12)1,65,600</td>
</tr>
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<td>ONE JRF</td>
<td>12000+1800HRA</td>
<td>(12)1,65,600</td>
<td>(12)1,65,600</td>
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<tr>
<td>TOTAL</td>
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<td>3,31,200.00</td>
<td>3,31,200.00</td>
</tr>
</tbody>
</table>

### 311. Justification of manpower requirement

These two research positions would be required to fulfill the objectives in the time frame committed in the proposal.
320. BUDGET FOR CONSUMABLE MATERIALS

<table>
<thead>
<tr>
<th>ITEM</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; year</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; year</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; year</th>
<th>TOTAL</th>
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<tbody>
<tr>
<td>1. Palsticware and reagents for cell culture</td>
<td>4,00,000</td>
<td>3,00,000</td>
<td>3,00,000</td>
<td>10,00,000</td>
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<tr>
<td>2. Biochemicals/ immunochemicals</td>
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<td>4,00,000</td>
<td>4,00,000</td>
<td>12,00,000</td>
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<tr>
<td>3. Protein sequencing &amp; generation of antibodies</td>
<td></td>
<td>4,00,000</td>
<td>5,00,000</td>
<td>9,00,000</td>
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<tr>
<td>TOTAL</td>
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<td>11,00,000</td>
<td>12,00,000</td>
<td>31,00,000</td>
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<td>FE</td>
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<td>22,039.60USD</td>
<td>24,043.20USD</td>
<td>62,111.80USD</td>
</tr>
</tbody>
</table>

321. Justification of costly consumable

As mentioned above, there would be use of cell culture during this study and that would require sterile plasticware in addition to reagents for preparing cell culture media. Biochemical and immunochemicals would be required for proteomic and Western blot analysis.

330. BUDGET FOR TRAVEL

<table>
<thead>
<tr>
<th>ITEM</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; year</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; year</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; year</th>
<th>TOTAL</th>
</tr>
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<tr>
<td>TRAVEL</td>
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<td>30,000</td>
<td>30,000</td>
<td>90,000</td>
</tr>
</tbody>
</table>

331. Justification of intensive travel, if any.

The travel money will be used for attending scientific meetings, and the contingency for covering publication costs and for small time repairing of instruments etc.
340. BUDGET FOR OTHER COSTS/ CONTINGENCIES

<table>
<thead>
<tr>
<th></th>
<th>BUDGET (in rupees)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1ST YEAR</td>
</tr>
<tr>
<td>Contingencies</td>
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<tr>
<td>Overhead</td>
<td>10,000</td>
</tr>
<tr>
<td>TOTAL</td>
<td>40,000</td>
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</table>

350. BUDGET FOR EQUIPMENT

<table>
<thead>
<tr>
<th>S No.</th>
<th>Generic Name of the Equipment along with make and model</th>
<th>Imported / Indigenous</th>
<th>Estimated costs (in foreign currency also)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Two dimensional electrophotresis system</td>
<td>Imported</td>
<td>Rs12,50,000 25,045USD</td>
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<tr>
<td>2.</td>
<td>Vertical gel apparatus, transfer apparatus and power supply</td>
<td>imported</td>
<td>Rs4,50,000 9,016USD</td>
</tr>
<tr>
<td>3.</td>
<td>Micropipettes</td>
<td>Imported</td>
<td>Rs2,00,000 4,007USD</td>
</tr>
</tbody>
</table>

351. Justification of the proposed equipment.

Two dimensional electrophoresis system, vertical gel apparatus, transfer apparatus and power supply will be required for proteomic and Western blot analysis. Currently, the P.I. shares a vertical gel apparatus with other investigators in the department.
410. Time schedule of activities through BAR diagram.

- Ordering of consumables, Recruitment of manpower.
- Sample collection and Standardization of Proteomics
- Identification and characterization of proteins from BALF and sera of patients and from cancer cell cultures.
- Analysis of antibodies in patients’ sera and lavages.
- Characterization of proteins obtained through proteomics and generation of antibodies, and probing of BALF and sera with these antibodies.
420. List of facilities being extended by parent institution(s) for the project implementation.

A) INFRASTRUCTURE FACILITIES:

<table>
<thead>
<tr>
<th>S.no</th>
<th>Infrastructural Facility</th>
<th>Yes/No/ Not required</th>
<th>Full or sharing basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Workshop Facility</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>2.</td>
<td>Water &amp; Electricity</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>3.</td>
<td>Laboratory Space/ Furniture</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>4.</td>
<td>Power Generator</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>5.</td>
<td>AC Room or AC</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>6.</td>
<td>Telecommunication including e-mail &amp; fax</td>
<td>Yes</td>
<td>Full</td>
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<tr>
<td>7.</td>
<td>Transportation</td>
<td>Yes</td>
<td>Sharing basis</td>
</tr>
<tr>
<td>8.</td>
<td>Administrative/ Secretarial support</td>
<td>Yes</td>
<td>Sharing basis</td>
</tr>
<tr>
<td>9.</td>
<td>Information facilities like Internet/ Library</td>
<td>Yes</td>
<td>Full</td>
</tr>
<tr>
<td>10.</td>
<td>Computational facilities</td>
<td>Yes</td>
<td>Full</td>
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<tr>
<td>11.</td>
<td>Animal/ Glass House</td>
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<td>-</td>
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</table>
B) Equipments available with the institute/ group/ department/ other institutes for the project:

 Equipments available with PI & his group

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Equipment/ Accessories</th>
<th>Make /Model</th>
<th>Funding Agency</th>
<th>Year of Procurement</th>
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<td>Thermocycler</td>
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<td>2.</td>
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<td>4.</td>
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<td>-86 ULT freezer</td>
<td>Daiham</td>
<td>do</td>
<td>2008</td>
</tr>
<tr>
<td>13.</td>
<td>Plant growth chamber</td>
<td>Daiham</td>
<td>do</td>
<td>2008</td>
</tr>
<tr>
<td>14.</td>
<td>LAF</td>
<td>Daiham</td>
<td>do</td>
<td>2008</td>
</tr>
<tr>
<td>15.</td>
<td>CO2 incubator</td>
<td>Sanyo</td>
<td>do</td>
<td>2007</td>
</tr>
</tbody>
</table>
Raies Ahmad, Ph.D.
Lal Baba Sahib, Zakura, Hazratbal, Srinagar, Kashmir, 190006, J&K, India.
Tel: (91-194) 2428211 (M) 09419001315; E-mail: raiess@mailcity.com

CURRENT APPOINTMENT:

Associate Professor
Department of Biotechnology*,
University of Kashmir,
Srinagar-190006, J&K India
* Ranked 6th in the top ten Biotech schools of India

Major responsibility: Teaching & Research
Other responsibilities: Administration

PROFESSIONAL EXPERIENCE

Assistant professor (1999-2007)
Division of sericulture and Centre for Biotechnology
Sher-e-Kashmir University of agricultural Sciences & Technology,
Srinagar Kashmir

Major responsibility: Teaching & Research

Project associate (1994-1998)
National Institute of Immunology
JNU Campus, New Delhi

Major responsibility: Research

EDUCATION:

- Ph.D. Faculty of Science; 2001
  (Worked for my PhD thesis at National Institute of Immunology JNU Campus, New Delhi)
  Thesis: “A study on the biochemical analysis and molecular characterization of silk proteins of Philosamia ricini”
Current projects in the laboratory

Project No.1

“IDENTIFICATION OF BRONCHOGENIC CANCER MARKER(S) THROUGH PROTEOMIC ANALYSIS OF BRONCHIAL ALVEOLAR LAVAGES AND SERA”

My group is involved with the mechanism responsible for EGFR over expression which is largely unknown, and gene amplification is only rarely observed in NSCLC. The lack of a consistent method of evaluating levels of EGFR has caused a disparity in reports of the EGFR as a prognostic factor. For some tumors, EGFR is a strong prognostic indicator associated with a more aggressive disease and reduced survival. HER (human epidermal growth receptor) family consists of HER1, HER2, HER3 and HER4. These receptors play important roles in the behavior of tumor cells by increasing proliferation, decreasing apoptosis, enhancing tumor cell motility and angiogenesis. Our study takes into consideration of all these EGFR family proteins and is looking for the expression of these proteins. This study may give us the idea to utilize these HER proteins as prognostic tools for the treatment of bronchogenic carcinoma. In this study we will investigate the role of epidermal growth factor receptor (EGFR) expression as a prognostic marker for predicting cancer behavior and clinical outcome in bronchogenic carcinoma patients undergoing potentially curative surgery.

Project No.2 (DST-funded project)

“ATTACHMENT AND GROWTH PROPERTIES OF SAFFRON CELLS CULTURED ON SILK PROTEIN MATRICES”

Cell culture techniques have been used to assess the biocompatibility or toxicity of medical devices and materials. The agar overlay method has been most widely utilized and is designed to detect the response of a monolayer cell culture to readily diffusible components from material applied to the surface of an agar layer overlaying the monolayers. Most of the work has been done on animal cell culture with biocompatibility of protein matrices and there are no reports on growth and attachment of the plant cell culture on protein matrices. Having medicinal effect, saffron is now used as drug against several diseases including cancer.
The proposed scheme is expected to develop a standard protocol for interaction of fibroin protein interaction with saffron tissue culture plants for rapid multiplication of cultivars which shall fulfill the huge demand of superior quality planting material.

RESEARCH EXPERIENCE:

Worked from 20th Jan.1995 to 30th Sept.1997 in the project entitled “Molecular Biological Studies of Silk Genes of Non-mulberry Silkworm” as Project Assistant in Eukaryotic Gene Expression Laboratory at National Institute of Immunology JNU Campus, New Delhi.

RESEARCH INTERESTS:

At the Sheri Kashmir Agriculture University, we were engaged in research on the developmental biology and immunology of silkworms. We have dissected and understood signals that lead to the induction of gene expression of silk proteins at various stages of larval development. These studies, in addition to giving important insights into the nature of extra cellular/intracellular signals that control the expression of these proteins, might also help in devising methods for increasing the expression of these commercially important molecules.

The second project which was also a thrust area in the laboratory deals with immunity in insects which have seen a sudden burst in the recent years. We were particularly interested in identifying and studying innate immune recognition molecules present in the silk worm as well as looked at signals that lead to generation of antibacterial peptide responses in these insects.

COLLABORATIVE AND OTHER RESEARCH PROJECTS

In addition to my thesis work, I was also involved in other projects, which were going on in the Eukaryotic Gene Expression Laboratory at the National Institute of Immunology (NII). These related to studies on heterologous expression of proteins in the baculovirus expression system and understanding various aspects of eukaryotic gene expression. I also worked in a collaborative research project between this laboratory at NII and Dr. Altaf Lal's laboratory at Centers for Disease Control, Atlanta, USA.

PARTICIPATION IN SUMMER INSTITUTES:

Worked at National Bureau of Plant Genetic Resource (NBPGR), New Delhi for three months on Chemical, Biochemical and Molecular characterization of plant genetic resource.
PUBLICATIONS:


Manuscript under preparation:

**Raies Ahmad**, Ayub Qadri. Analysis of antibody response to antigens of *Salmonella typhi*.

SEMINARS/CONFERENCES ATTENDED:


Ph.D. and M. Phil. Students:

- Qazi Danish Mushtaq
- Shoiab Bukhari
- Taseem Mokhdomi

NATIONALITY:

INDIAN

REFERENCES:

Dr. Seyed E. Hasnain, FNA, FASc, FNASC, FTWAS
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Hyderabad, India
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Product Development Cell
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JNU Campus, New Delhi-110067
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Dr. Rahul Pal
Senior Scientist
Imunoendocrinology Laboratory
National Institute of Immunology
JNU Campus, New Delhi-110067
E-mail: rahul@nii.res.in
Tel: 91-011-26703562
Co-Investigator
Dr. Rafi Ahmad Jan
Hakim Bagh, Rawalpora, Sanat Nagar,
Srinagar, Kashmir-190005, J&K, INDIA.
Tel: +91-194-242301 (R) +91-9419009374 (cell)
e-mail: rafi@yahoo.com

CURRENT APPOINTMENT:
Associate professor, Internal Medicine,
SKIMS, Soura, Srinagar-190011
J&K, INDIA.

EDUCATION:

UNDER GRADUATE MEDICAL CAREER:

Medical School (MBBS) kakatiya Medical College
Warangai, Hyderabad.
Date of Entry: 1979
Date of Completion 1984
Internship Training Govt. Medical College and Associated Hospitals
Srinagar, Kashmir.

POSTGRADUATE MEDICAL CAREER:

M.D (Internal Medicine) S K Institute of Medical Sciences, Soura,
Srinagar, Kashmir.
450. Details of research projects being implemented/ completed/ submitted by the investigator(s)/ Co-investigator(s).

<table>
<thead>
<tr>
<th>Title of Project</th>
<th>Cost (in Rupees)</th>
<th>Status</th>
<th>Agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Attachment and Growth Properties of Saffron cells cultured on Silk protein Matrices.</td>
<td>30,00,000</td>
<td>Approved and Operational</td>
<td>DST</td>
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<tr>
<td>2. Exploring the potential of consumable herbs for Kashmir region as a source of signal transduction modulators (STMs) to serve as modern drug leads.</td>
<td>43,10,000</td>
<td>-do-</td>
<td>ICMR</td>
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<td>3. Proteome Profile of heat shock proteins in human esophageal cancer.</td>
<td>6,42,000</td>
<td>-do-</td>
<td>ICMR</td>
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<td>4. SNP analysis of candidate genes associated with high myopia in ethnic Kashmiri Pedigrees.</td>
<td>10,70,000</td>
<td>-do-</td>
<td>Ministry of Science and Technology, Biotech Division</td>
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