

ACTREC'S OPEN DAY 2009

3 - 4 December 2009



Since 1995, the Cancer Research Institute (CRI, then located on the Tata Memorial Centre's Parel campus) has been holding an 'Open Day'. This tradition has continued, after a break in 2002, even after CRI shifted to the newly established 60-acre campus of the **Advanced Centre for Treatment, Research and Education in Cancer (ACTREC)** in Kharghar, Navi Mumbai. **ACTREC's Open Day 2009 has been scheduled for Thu. 3rd and Fri. 4th December 2009.**

Over a two day period, around 500 students and their faculty from over 50 science degree colleges of Mumbai and Navi Mumbai will visit ACTREC. Posters on cancer awareness, prevention, research and treatment strategies will be displayed prominently in the entrance lobby. At the start of the visit, an Introductory Talk covering the thrust areas of basic and clinical research on cancer at the Centre will be delivered to the students. Batches of 12-15 students from each college, accompanied by their teachers, will be taken on visits to different PI labs/ facilities/ departments of the basic and clinical research wings. In CRI, scientists and Ph.D. scholars will give poster-aided demonstrations of latest techniques and protocols used in biomedical research in cancer, *vis a vis* their on-going research projects. In the Clinical Research Centre (CRC), clinicians and technologists will demonstrate high end equipment and facilities used for cancer diagnosis and treatment.



The following lab visits will feature in Open Day 2009: Animal House, Common Instrument Room, Laser Confocal Microscope, Sorab Lab, Chilakapati Lab, Kalraiya Lab, Chiplunkar Lab, Vaidya Lab, Radiation Oncology and Transfusion Medicine. Brief write-ups on these lab demonstrations are given below.

1 ANIMAL HOUSE

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Animal Models in Cancer Research

The main activity of the Animal House (AH) is breeding, maintenance and supply of laboratory animals to institutional researchers against animal study proposals sanctioned by the Institutional Animal Ethics Committee (IAEC). The AH breeds 18 different strains of mice including Nude, SCID, transgenic and knock outs; SD strain of rat; 2 strains of hamsters: and one rabbit strain. Since Nude and SCID mice lack T-cells, these mice are bred and maintained in an Individually Ventilated Caging (IVC) system. Some normal mouse strains are also maintained under IVC. The AH has programs to monitor animal food/ water quality; microbiological/ parasitological (clinico-pathological) infestation and genetic purity (skin grafting, biochemical and SNP markers) of the animals. The AH also monitors T- and B-lymphocyte profile of Nude and SCID mice to ascertain the integrity of these animals. AH also provides quality control services, viz. ELISA tests, for detecting the presence of rodent pathogens and biochemical marker tests to check strain purity. This facility also supplies breeders of available strains

to CPCSEA-registered animal facilities in the country. [For details about the AH and available animal strains, visit: http://www.actrec.gov.in/animal_main.htm].

During Open Day 2009, the vital importance of animal models in cancer research will be highlighted through a display cum demonstration of representative strains of mice, rat and hamsters, detailing their strain characteristics and specific application for various kinds of studies, for the visiting students.

2 COMMON INSTRUMENT ROOM

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Instrumentation in Biological Research

ACTREC maintains centralized Common Instrument Rooms (CIR) in order to optimize the utilization of all major general as well as high end scientific instruments, and to make them available to all the staff and students of the centre. Equipment such as spectrophotometers, high speed and ultracentrifuges, HPLC, gamma counter, Elispot reader, etc., are available for use round the clock on all days, including holidays. A team of qualified technical staff attached to the CIR handles routine maintenance and renders help to ensure proper usage of the equipment.

During this year's Open Day, the following equipment having wide utility in biological research - for general use as well as for specific applications/ assays, will be demonstrated to the visiting students:

- Thermal cycler
- Gel Documentation system
- Dual beam spectrophotometer
- Microplate absorbance reader
- High speed Centrifuges
- Preparative Ultracentrifuge
- Sonicator

3 LASER CONFOCAL MICROSCOPE

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Laser Confocal Microscope (LCM) - Applications in Cancer Research

Seldom has the introduction of a new instrument generated as instant an excitement among biologists as the laser scanning confocal microscope (LCM). With this microscope, one can slice incredibly clean,

thin optical sections out of thick fluorescent specimens; view specimens in planes tilted to, and even running parallel to, the line of sight; penetrate deep into light scattering tissues; gain impressive three dimensional (3D) views at very high resolution; obtain differential interference or phase contrast images in exact register with confocal fluorescence images; and finally improve the precision of microphotometry. How does it achieve that? In laser confocal microscopy (LCM), illumination of a single point in a specimen is combined with imaging of this single illuminated point by a point detector. In short, only the 'in focus' object is visualized while light coming from 'out-of-focus' planes is rejected, resulting in an image with better resolution. Point illumination is achieved by using a laser as the source of illumination, while point detection is achieved using confocal aperture. LCM enables optical sectioning of the object and building a three-dimensional (3D) image. The image is detected using sensitive detectors like photo-multiplier tubes (PMT) or photo-diodes. Optical sectioning is done either by moving the focal point of the laser, or by moving the object on the stage using a z-motor. Optical sectioning minimizes sample-processing time and enables the observation of deep-seated, weak intracellular signals. Use of multiple PMT allows studying multiple parameters simultaneously. LCM has extensive cell biology applications which include studies of cellular organelles (nucleus, mitochondria), cellular signaling events (Ca^{++} release, pH), physiological processes (cell motility, cell division), live cell imaging (drug uptake, cell-cell interaction), co-localization of various molecules, etc.

4 SORAB LAB

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Proteomics of Cellular Pathways

Our laboratory is interested in understanding the mechanisms by which the 14-3-3 family of proteins regulates pathways that are required for cell cycle arrest in response to DNA damage. Our previous results show that a decrease in the expression of one 14-3-3 isoform, 14-3-3 γ , leads to an override of a DNA damage induced cell cycle arrest and cell death. To identify novel ligands for 14-3-3 γ that might play a role in regulating these pathways, we have designed a novel proteomic screen to identify novel 14-3-3 γ ligands. These experiments have led to the identification of ligands by mass spectrometry resulting in an increased awareness of how this important group of proteins regulates cell cycle progression in response to DNA damage and how this could be exploited for tumor therapy.

The other focus of this laboratory is to determine the role of plakophilin3 in regulating desmosome assembly and neoplastic progression in epithelial cells. Our lab has previously demonstrated that

downregulation of plakophilin3 leads to neoplastic progression. We also observed that the neoplastic progression associated with plakophilin3 loss is accompanied by an increase in the expression of cytokeratin 8 and 18. A real time PCR analysis revealed that the increase in the expression levels of cytokeratin 8 and 18 is due to alteration in the protein stability and not due to increased mRNA transcript levels. Post translational modifications have been reported to regulate the stability of cytokeratin 8. To determine whether increased stability of cytokeratin 8 in the cells that lack plakophilin3 is due to differential post translational modifications, 2-D gel electrophoresis followed by MALDI-TOF was performed. The 2-D profile analysis demonstrated altered migration of cytokeratin 8 in the cells lacking plakophilin3 as compared to the vector control suggesting that cytokeratin 8 is differentially modified upon plakophilin3 loss. Identification of the post translational modifications on cytokeratin 8 will lead to novel insights into how this protein regulates the process of neoplastic progression.

5 CHILAKAPATI LAB

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Diagnosis of Cancers by Raman Spectroscopy

Screening is an important tool in the overall management of cancer and in reducing cancer burden in the population. The prognosis of almost all cancers depends on the stage of diagnosis. Therefore detecting the disease in the early or precancerous stages is likely to be beneficial in terms of improving the outcome. However for many cancers there are no specific screening methods as tests that are reasonably sensitive and specific while at the same time being cost effective and reproducible are yet to be developed for these cancers. Optical spectroscopy (IR absorption, Raman scattering fluorescence) are being actively pursued as potential alternatives/ adjunct methods to existing techniques in various aspects of cancer management, which includes screening. Among them, Raman spectroscopy (low water absorption and less harmful NIR excitation) is more suited for *in vivo* applications. This technology consists of illumination of the mouth cavity with a special, non hazardous light and then studying the scattered light of the mucosa. Our *ex vivo* studies have demonstrated the efficacy of these technologies in classifying normal, inflammatory, oral sub mucous fibrosis (OSMF) and malignant conditions. Presently we are pursuing a translational approach.

Electrophoresis and Western Blotting

Electrophoresis and Western blotting techniques are used to characterize the proteins with respect to the purity their size, subunits, isoelectric point and finally identity with respect to reactivity with specific antibody. SDS-PAGE is the most widely used technique, which analyzes the size and subunits of the proteins. Isoelectric focusing separates them on the basis of their isoelectric point. When used together as 2D-Gel electrophoresis, it is able to resolve several hundreds of proteins. It is most effective in confirming the purity of the proteins. Western blotting is most effective in confirming the identity of the proteins. Proteins separated on SDS-PAGE are transferred electrophoretically either on to nitrocellulose or PVDF membranes, and probed with specific antibodies. The antibodies are tagged with enzymes like horse radish peroxidase or alkaline phosphatase. The substrates for these enzymes that either give chromogenic or fluorescent products are used to visualize specific proteins on the membranes.

Enzyme Linked ImmunoSorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) - a technique developed by two Swedish scientists Engvall and Perlmann in 1971, is used for detecting the presence of specific substances such as enzymes, viruses, bacteria, or antibodies in biological samples. This technique is widely used as a diagnostic tool to detect the presence of pathogens (hepatitis virus, human immunodeficiency virus, etc), tumor markers (alpha feto protein, carcino embryonic antigen, prostate specific antigen, etc) and cytokines in biological samples. It is a method of choice because of its sensitivity, simplicity and speed, and the added advantage is that it does not use radioactive compounds.

The ELISA technique involves immobilization of the antigen on microtiter plates, addition of sample in which the antibody to the immobilized antigen is present, followed by addition of a secondary antibody conjugated to a suitable enzyme (e.g. horse radish peroxidase). Color development occurs after the appropriate substrate (ortho phenylene diamine) is added. The optical density can be measured at the specific wavelength using an ELISA reader. Various modifications of this standard protocol are available - sandwich ELISA, competitive ELISA and cell based ELISA. A newer application of this technique is the enzyme-linked immunosorbent spot (ELISPOT) assay. ELISPOT assays were originally developed to enumerate B cells secreting antigen-specific antibodies, and have subsequently

been adapted for the identification and enumeration of cytokine-producing cells at the single cell level. The ELISPOT assay provides both qualitative (type of immune protein) and quantitative (number of responding cells) information, and is used to monitor the functional dynamics of immune cells.

8 VAIDYA LAB

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ImmunoHistoChemistry

Immunohistochemistry (IHC) is an important application of monoclonal or polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease. Immunohistochemistry is widely used for the diagnosis of cancers, because specific tumor antigens are expressed *de novo* or are upregulated in certain cancers. The application is used routinely in validation of disease targets as it allows us to visualize the expression of a target in the affected tissue during the disease process. Immunohistochemistry requires the availability of biopsies; these are processed into sections with a microtome, then the sections are incubated with the appropriate antibody. The site of antibody binding is visualized under an ordinary or a fluorescent microscope using a marker such as a fluorescent dye, enzyme, radioactive element or colloidal gold that is directly linked to the primary antibody or to an appropriate secondary antibody.

Our laboratory is working on cytokeratin expression in human oral pre cancer and cancer, and its possible use as early diagnostic/ prognostic marker. We have been able to find differences in CK expression and localization between normal, premalignant and tumour tissues using IHC.

9 RADIATION ONCOLOGY

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Helical TomoTherapy

Radiation therapy has been an established modality for the treatment of cancer. Rapid advancements in technology allow planning and delivery of highly conformal doses to the target tissues while minimizing doses to surrounding normal critical structures. Helical TomoTherapy is one such novel and integrated platform for planning, verification, and delivery of Intensity Modulated Radiation Therapy (IMRT). In Helical TomoTherapy, a linear accelerator is mounted in ring-gantry configuration like a computed tomography (CT) scanner. The linear accelerator continuously rotates around the patient as the couch is translated through the ring-gantry delivering radiation in a spiral or helical fashion. The fan-beam is highly modulated by 64-pairs of very fast moving binary multi-leaf collimators (MLCs). The computer-controlled MLCs have two sets of interlaced leaves that move in and out very quickly to

constantly modulate the continuous radiation beam as it leaves the accelerator, while the couch is also translating through the bore. An array of CT detectors opposite the beam line allow a verification CT scan to be performed after positioning prior to every single fraction, which is then co-registered with the planning CT. Any discrepancy in the planned position and treatment position (both translational and rotational) is corrected by applying the shifts. The helical delivery coupled with volumetric in-room image-guidance highly increases the precision and conformity of radiation, thereby improving the therapeutic ratio. Helical TomoTherapy is an important value-addition to the growing armamentarium of the radiation oncology community practicing high-precision conformal radiotherapy.

10 TRANSFUSION MEDICINE

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Blood Banking

The department of Transfusion Medicine (DTM) at ACTREC provides services of blood collection from voluntary donors, processing the collected blood into components, plateletpheresis, as well as stem cell collection, processing and storage. Special attention is given to the quality and sterility of blood and blood components that are supplied to patients admitted not only at ACTREC but also at other hospitals and nursing homes. DTM also meets the requirements for specialized blood products such as leucoreduced, gamma irradiated and single donor platelets for treating transplant patients. DTM has successfully met the rising demands for blood and blood components by conducting regular blood donation camps and maintaining a voluntary blood and platelet pool. The department has the following sections: Area for Blood Donation and Apheresis, Area for Medical & Physical examination and Donor Counselling, Red Cell Serology laboratory, Transfusion Transmitted Infections testing laboratory, Blood Component laboratory, and Quality Control laboratory. DTM has three cell separators [apheresis devices - Comtec (Fresenius), Amicus (Baxter) and Cobe Spectra (Gambro)] that enable plateletpheresis, peripheral blood stem cell harvest as well as therapeutic apheresis procedures to be performed here. A blood irradiator (BI – 2000) is used for gamma irradiation of all cellular blood products issued to medical oncology patients. Specialized blood products like leucocyte depleted packed red cells and platelets are also available. Issue of blood and blood products, and apheresis for harvesting platelets from donors are carried out routinely around the clock in DTM.