DEVELOPING A PRIMARY CULTURE MODEL FROM CERVICAL CANCER TISSUES CAPABLE OF PERFORMIMG FUNCTIONAL ASSAYS AND PREDICTING RADIO-SENSITISATION

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Introduction

- Cancer of cervix is the leading cancer in India after breast cancer and it contributes to the 25% global mortality rate.
- Cervical cancer is mainly caused by the persistence infection of high risk-human papilloma virus (HR-HPVs).
- The tumor development and progression in cervical cancer is triggered by the sustained expression of the two viral oncogenes E6 and E7.
- The primary treatment includes surgery in early stage and chemo radiation in advanced stage. However, in advanced cervical cancer, about 50-60% woman respond to radiotherapy while others do not.
- Chemo-radio resistance is the frequent event for cervical cancer. Therefore, we need a primary model to predict chemo-radio resistance. But the existing methodology enriches fibroblastic population in pre-clinical model which is not suitable for functional studies.

Aims and objectives

- To build a pre-clinical model for doing functional studies in cervical cancer enriching epithelial population.
- To develop a tool to predict chemo/radio-sensitization/resistance from the tumor tissues.

Methodology

Collection of cervical cancer tissue

Operation theatre(OT)or from punch biopsy in outdoor patient clinics

Processing of the tissue for primary culture

- In the previous method (old method), collagene II was used to digest the tissues.
- Collect the tumor punch from cervical cancer patients
- Wash with PBS and transfer to 15 ml centrifuge tube in Dispase II solution (1.6U /ml, for 2-3 ml)
- Keep at 4^0C for 16hr followed by 1 hr room temperature
- Collect the Dispase II solution and wash with PBS
- Transfer the tissue in 0.025% trypsin- EDTA solution, dissected into, ~3mm pieces
- Keep 12 min at 37^0C with gentle shaking
- Neutralized the solution with equal volume DMEM (10% FCS)
- The cell suspension will be transferred to a 15ml tube, centrifuged at 400xG for 5 minutes.
- PBS wash, re-suspended in full medium KSFM (5% FCS) and will be placed in a T25 flask.
- Keep in CO_2 incubator and change the medium

- **Homologus Recombination (HR) Assay**

Fig.5.

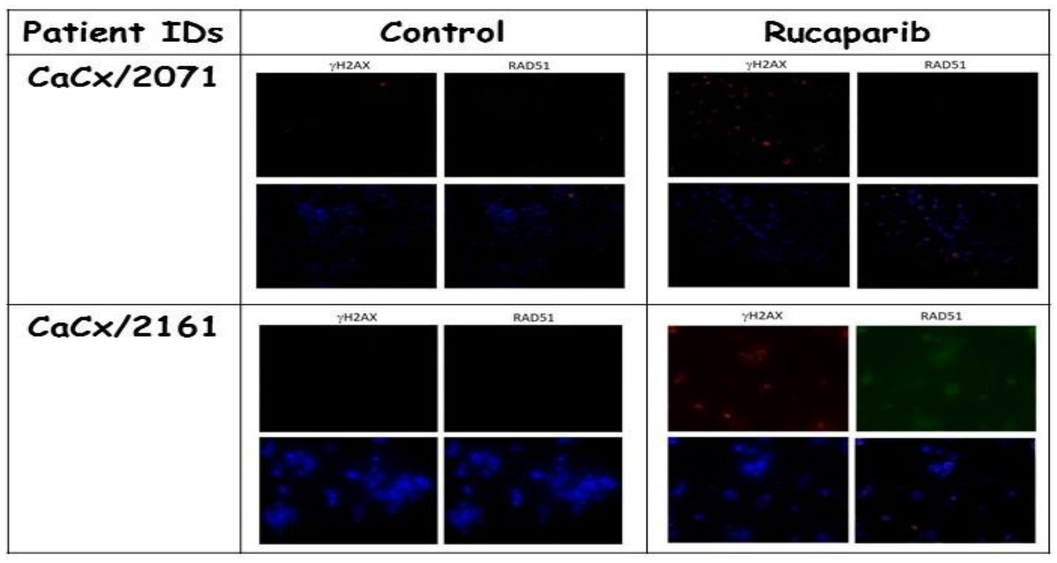

Fig.5. Effect of Rucaparib in homologus recombination repair assay in cervical cancer patients

Results

Characterisation

- **Development of primary culture**

Fig. 1.

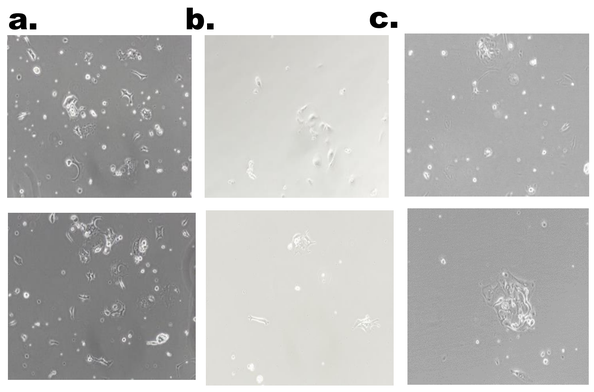

Fig.1.The figure depicts the very less attachment of primary cultured cells of different patients (a. CaCx/2081; b. CaCx/2161; c. CaCx/2033) using the old methodology.

Fig. 2.

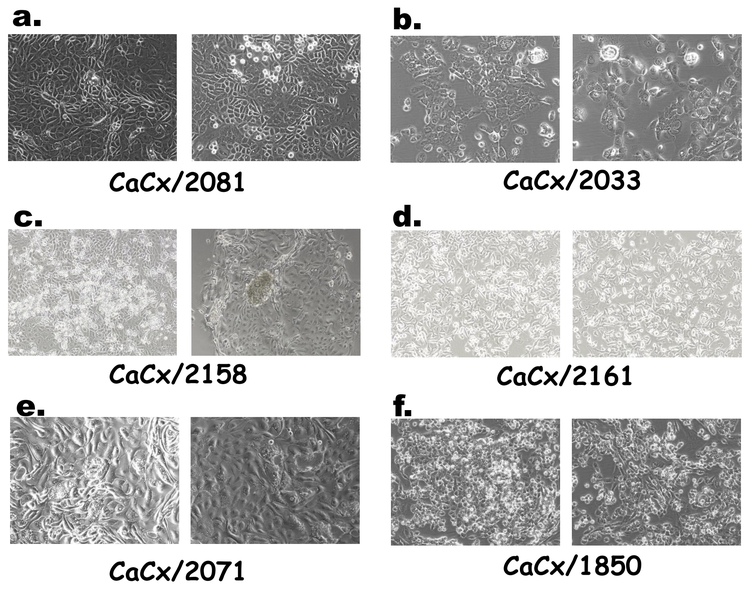

Fig.2. Typical cobblestone like structure is seen in the primary culture of different patients which is indicating primary epithelial cells using modified methodology.

- **Immunofluroscence assay**

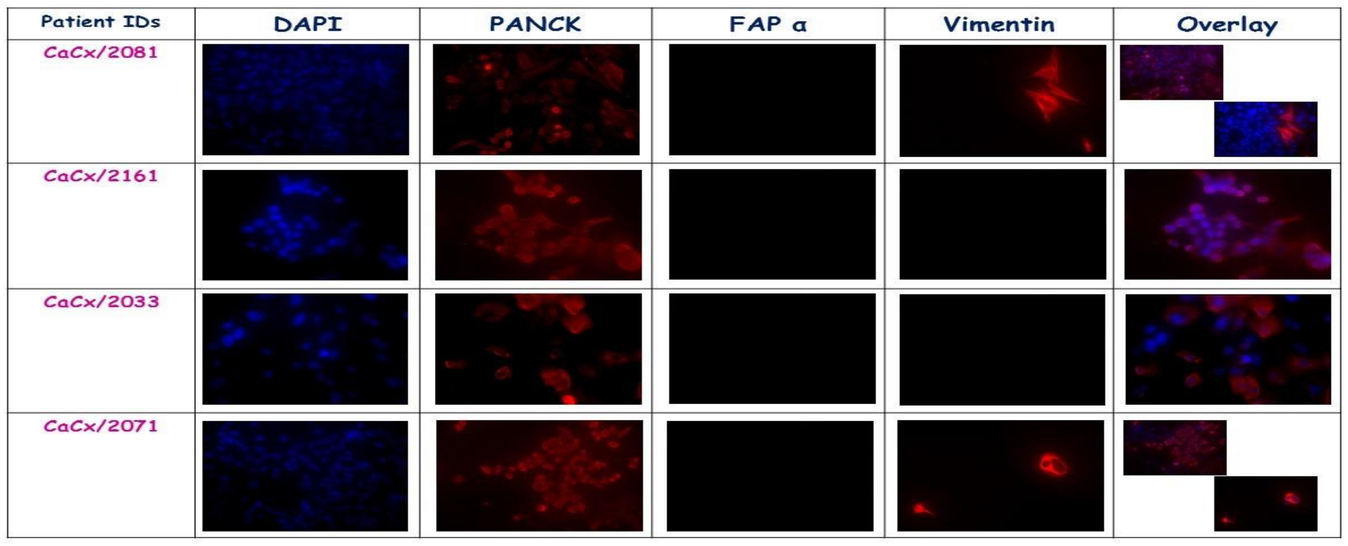

Fig.3. Cells stained with different IF markers like PANCK, FAP α, Vimentin to evaluate the enrichment of epithelial cells

Functional Assay

- **Cytotoxicity Assay (MTT)**

Fig.4a.

	CaCx/2161	CaCx/2071	CaCx/2081
IC 50 Cisplatin	10.6597	10.6596	24.3609
IC 50 Bleomycin	5.22	5.29	>100

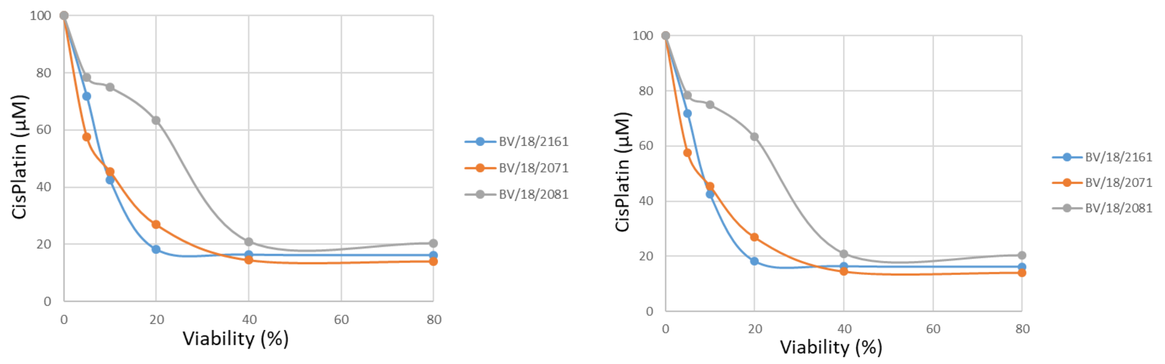

Fig.4b.

Patient ID		Bleo	Ru10 +Bleo	CPT5+ Bleo	SMAR10 +Bleo
CaCx/2071	IC50	5.29	5.62	5.12	4.87
CaCx/2161	IC50	5.55	5.94	5.64	5.04
CaCx/2081	IC50	>100	>100	>100	>100

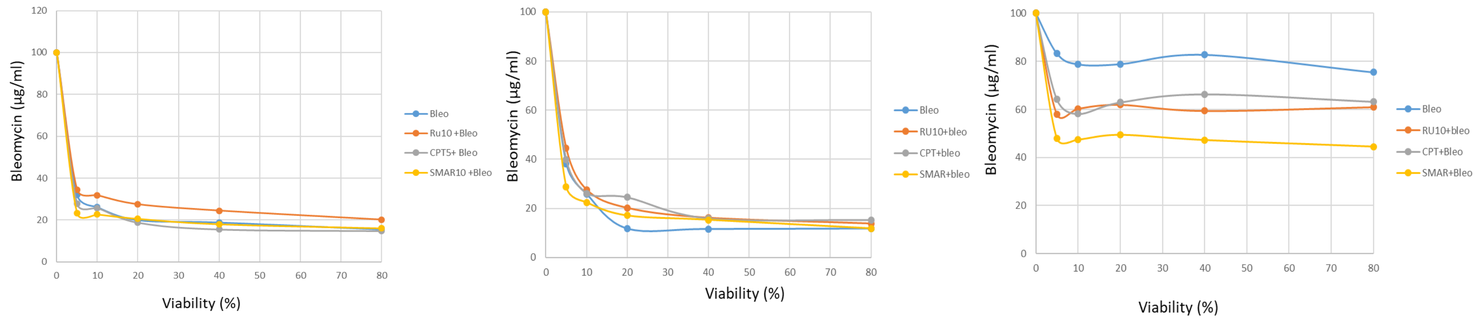

Fig.4. In the above figure (a) depicts cytotoxicity with Cisplatin and Rucaparib; (b) depicts cytotoxicity with Bleomycin in combination with other drugs

Future Directions

- Other drugs screening assay in the cervical cancer related to radioresistance
- Development of PARP activity assay to correlate with the chemosensitization/radiosensitization
- To develop Non-homologus end joining (NHEJ) assay to check for repair pathways for the patients

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