VAISHALI MULCHANDANI

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Curriculum vitae

Present Status

5th year Integrated Ph.D. Scholar (MS-PhD), Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata, India (Master's CGPA: 8.64) (2017-Current)

Under the Supervision of Prof Jayasri Das Sarma, Department of Biology, IISER Kolkata.

Educational Qualifications

Degree/Certificate	School/College	Board/University	Year of Passing	Percentage/CGPA
Int.Ph. D Semester 6 (Masters)	Indian Institute of Science Education and Research Kolkata	Indian Institute of Science Education and Research Kolkata	2019	8.64 CGPA
B.Sc. Microbiology (H)	Institute of Home Economics	University of Delhi	2017	73.5%
AISSCE (12th)	Carmel Convent School, New Delhi	CBSE Board Exam	2014	77.6%
SSE (10th)	Carmel Convent School, New Delhi	CBSE Board Exam	2012	8.2 CGPA

Research Interest

- Cancer Biology
- Molecular Biology
- Cell Signaling

Research Experience

- Senior Research Fellow (2021-present) and Junior research fellow (2019-2021) at IISER Kolkata
- Collaborative research work at Newcastle University, United Kingdom from 1st August 2019 to 29th November 2019 under Dr. Nicola Curtin on 'Hyperthermia and immune modulation in homologous recombination stratified epithelial ovarian cancer: Development of targeted therapeutic approaches' under the DST-UKIERI.

- Research project on "Assessment of indoor aeromycoflora from schools of Delhi" at University of Delhi (2015)
- Rotator internship in microbiology department of Deen Dyal Upadhayay Hospital, New Delhi from 9th Dec. 2014 to 23rd Jan. 2015

Achievements

- Qualified Joint Admission Test for M.Sc., (JAM) in Feb 2017
- Qualified GS2017 – JGEEBILS/ TIFR Biology Entrance Test in 2017

Publications

Book Chapter

- Saurav*, Vaishali Mulchandani* and Jayasri Das Sarma. Interplay between redox homeostasis and oxidative stress in the perspective of ovarian and cervical cancer immunopathogenesis. (In press)

Paper

- A Bose, M Maulik, V Mulchandani, D Thomas, P Mukherjee, M Koval and J Das Sarma. ERp29 attenuates coronavirus replication and infectivity. (Under communication)

Poster Presentation

- Poster presentation in "Frontiers in Modern Biology (FIMB) 2020" at Indian Institute of Science Education and Research – Kolkata from 28th to 29th February, 2020.
- Poster Presentation at 1st Annual Meeting of Kolkata Gynecological Oncology Trials and Translational Research Group (KolGOTrg) from 2nd -4th March,2019

Teaching Experience

- Teaching Assistant for Spring 2021 semester at IISER-Kolkata for the course 'Cloning & Protein Expression Laboratory' under Dr. Jayasri Das Sarma.
- Teaching Assistant for Autumn 2020 semester at IISER-Kolkata for the course 'Physiology and Developmental Biology Lab' under Dr. Malancha Ta.
- Teaching Assistant for Spring 2020 semester at IISER-Kolkata for the course 'Cloning and Protein Expression Laboratory' under Dr. Jayasri Das Sarma.

Workshop & Symposiums attended

- Participated in the Bilateral Indo-US Webinar on COVID Biology organized by IISER-Kolkata in collaboration with IISc, Bangalore, University of Pennsylvania-USA, and University of Colorado, USA , Between 16th – 19th August, 2020.
- Participated in the "New insights into the Inflammation, Immunity, and Pathobiology of Diseases" Indo-US Symposium organized by Department of Biological Sciences, IISER, Between 3rd – 8th December, 2019.
- Participated in course "The prevalence of Antibiotic Resistance in the Environment" conducted by Professor Sharon Gusky under the 2018 ASM-IUSSTF Indo-US Teaching Programme from January 3-4 and January 7-10, 2019.
- Attended a workshop on "Advanced bioanalytical methods and applications & Overview of Outreach activities in advanced microbiology education and teaching" in Gangtok from 24 February to 1st March, 2018.
- Participated in "Frontiers in Modern Biology (FIMB) 2018" at Indian Institute of Science Education and Research – Kolkata from 19th to 21st January, 2018.
- Attended a week-long hands-on training programme in Molecular Biology Techniques at Agri Biotech Foundation, Hyderabad in May-June 2016.
- Attended a hands-on workshop on Bioinformatics in January-2016.
- Attended a 2-day hands-on training workshop on "Cell Culture Techniques and

Cytotoxicity Assays" at Amity University, Noida in October-2015.

- Participated in the Annual Microbiology Festival and Symposium on "Biofuels: An Alternative and Non-conventional Energy Source for Future, at Bhaskar Acharya College of Applied Sciences in 2015
- Attended a Science Setu workshop on "Stem cell science and applications- hype or reality" organized by National Institute of Immunology in September, 2015.
- Participated in the SYSCON-2014 on "Recent Advances in Biological Sciences" at AIIMS, New Delhi

Other Skills

- Cell Culture/ Tissue Culture
- Animal Dissection, maintenance and breeding
- Immunofluorescence
- Immunoblotting
- PCR
- Cytotoxicity assays (Clonogenic assay, MTT assay, SRB assay)
- Gene Cloning and expression
- Microscopy
- Dye Transfer Assay
- Basic knowledge of Computers (M.S. Office)
- GraphPad Prism
- ImageJ
- Adobe Photoshop
- Adobe Illustrator

Extra- curricular

- Got first position in "Science Hunt competition" organized by the life sciences department of the Ramjas College, University of Delhi (February 2016).
- Won Best Poster Competition in B.Sc. (H) I-year.
- Won second prize in Quiz Competition in Inter-college fest "Quanta-innovation-2015" at Daulat Ram College, University of Delhi
- Special merit certificate for "Best Poster Presentation" held at Institute of Home Economics in 2015
- Special merit certificate for consolation in "Quiz" held at Institute of Home Economics in 2015
- Merit certificate for participating in " My Dream Research Proposal" held at Institute of Home Economics in 2015
- Got the third prize in "Play the Game" event in the Annual Microbiology Festival and Symposium on "Biofuels: An Alternative and Non-conventional Energy Source for Future, at Bhaskar Acharya College of Applied Sciences in 2015
- National level chess player and Collage chess team captain for 3 years

भारतीय विज्ञान शिक्षा एवं अनुसंधान संस्थान कोलकाता

INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH KOLKATA

IISER KOLKATA | Major: **Biological Sciences** | **Integrated PhD Programme** | Medium of Instructions: **English**

Transcript of Academic Records for Vaishali Mulchandani, Roll No: 17IP003

Semester: I — Session: 2017-18

Code	Course Title	Type	Credit	Grade
LS3101	Immunology	T	4.0	A
LS3102	Cell Biology	T	4.0	A
LS3103	Microbiology	T	4.0	A
LS3104	Biology Laboratory V	L	4.0	A+
LS3105	Biology Laboratory VI	L	4.0	B
LS3106	IPhD Laboratory Rotation I	P	4.0	A
LS4105	Neurobiology	T	4.0	A

Total semester credit: 28.0 SGPA: 8.86 **CGPA**: 8.86

Semester: II — Session: 2017-18

Code	Course Title	Type	Credit	Grade
LS3201	Ecology and Conservation	T	4.0	B+
LS3202	Gene Regulation and Cellular Communication	T	4.0	B+
LS3203	Biophysics II	T	4.0	B
LS3205	Biology Laboratory-VIII	L	4.0	A
LS3206	IPhD Laboratory Rotation II	P	4.0	B
LS4205	Bioinformatics	T	4.0	A+
LS4208	Marine Biology	T	4.0	A

Total semester credit: 28.0 SGPA: 8.29 **CGPA**: 8.57

Semester: III — Session: 2018-19

Code	Course Title	Type	Credit	Grade
ID4107	Chemical Perspectives of Biological Pathways	T	4.0	A
LS4101	Plant Biology	T	4.0	B+
LS4102	Physiology	T	4.0	A
LS4103	Developmental Biology	T	4.0	B+
LS4107	IPhD Second Year Project I	P	4.0	A

Total semester credit: 20.0 SGPA: 8.6 **CGPA**: 8.58

Semester: IV — Session: 2018-19

Code	Course Title	Type	Credit	Grade
LS4201	Structural Biology	T	4.0	B+
LS4203	Advanced Biochemistry and Cellular Metabolism	T	4.0	B
LS4204	Cancer Biology	T	4.0	A+
LS4206	IPhD Second Year Project II	P	4.0	A
LS4207	Biology Lab X	L	4.0	A

Total semester credit: 20.0 SGPA: 8.6 **CGPA**: 8.58

Semester: V — Session: 2019-20

Code	Course Title	Type	Credit	Grade
LS5104	IPhD Third Year Project I	P	8.0	B+

Total semester credit: 8.0 SGPA: 8.0 **CGPA**: 8.54

Semester: VI — Session: 2019-20

Code	Course Title	Type	Credit	Grade
LS5202	IPhD Third Year Project II	P	8.0	A+

Total semester credit: 8.0 SGPA: 10.0 **CGPA**: 8.64

Verified by

Date: February 8, 2021

Assistant Registrar (Academic)

Attested

Dean of Academic Affairs

Interplay Between Redox Homeostasis and Oxidative Stress in the Perspective of Ovarian and Cervical Cancer Immunopathogenesis

Saurav Kumar, Vaishali Mulchandani, Anurag Banerjee, and Jayasri Das Sarma

Contents

Abstract

Generation of reactive oxygen species (ROS) is an inevitable part of cellular metabolism that is controlled by the antioxidant defense mechanism to maintain redox homeostasis. Under external or internal stress, this redox balance gets perturbed leading to high ROS levels and thus oxidative stress. Persistent oxidative stress contributes to the neoplastic transformation of healthy tissues. Oxidative stress is a crucial factor in the process of carcinogenesis as it aids in the initiation, promotion, and progression of tumor cells. ROS stimulates the growth of cancer cells by regulating various signaling pathways and suppressing antitumor immune responses. Tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) present in the tumor inflammatory

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S. Chakraborti et al. (eds.), *Handbook of Oxidative Stress and Cancer*,
https://doi.org/10.1007/978-981-15-4501-6_69-1

microenvironment utilize ROS to inactivate antitumor T-cell activity. Ovarian and cervical cancer are the most lethal gynecological cancers that are associated with inflammation related to oxidative stress. The standard medications for ovarian and cervical cancer treatment are surpassed by the tumor cells leading to more aggressive cancer phenotypes. The modulation of ROS levels by the action of chemotherapeutics along with immunotherapy is a potential strategy to cure cancer patients. The use of T-cell based therapy is a well-known approach for treatment of cancer. However, the efficacy of treatment is hampered by the functional inactivation of T cells when these cells enter into highly oxidative tumor microenvironment. Thus, to increase the efficacy of T-cell based therapy, the use of antioxidants to decrease the ROS levels, can prove to be a potential strategy to treat cancer.

Keywords

Reactive oxygen species (ROS) · Reactive nitrogen species (RNS) · Oxidative stress · Redox signaling · Antioxidant defense · Tumor microenvironment · Chronic inflammation · Immunosuppression

Introduction

Generation of reactive oxygen species (ROS) like superoxide (O^{-}_{2}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) are common to several cellular and immunological signaling pathways, synthesis of cellular structures, and host's defense against pathogens. However, uncontrolled ROS production can cause cellular damage that is implicated in a variety of pathological and age-related altered physiological conditions. Redox biology and oxidative stress are the two facets of ROS. While redox biology accounts for a minor increase in ROS levels that regulate signaling pathways to initiate biological processes, oxidative stress denotes uncontrolled ROS generation that damages DNA, protein, or lipids. Elevated production of ROS, if not balanced by the antioxidant defense, may lead to consecutive alteration in the intracellular homeostasis and cause damage to cellular components (Schieber and Navdeep 2014). Excessive ROS generation and accumulation is detected in almost all cancers, where they promote many aspects of tumor initiation, promotion, and progression. This chapter will discuss the current established knowledge of the effector function of ROS in the development of two most lethal gynecological cancers, i.e., ovarian and cervical cancer and cellular immunity.

Ovarian cancer is the most lethal and third most common cancer among all other gynecological malignancies (Bray et al. 2018). This disease is designated as a silent killer because of its asymptomatic tumor growth in the early stages. The symptoms arise in advanced stages of cancer resulting in late diagnosis and poor survival. More than 90% of ovarian cancer cases are epithelial in origin with five different histological subtypes (Prat 2012). Ovarian carcinoma cells tend to sustain a prooxidant state by overexpressing key prooxidant enzymes including NADPH oxidases

(NOX), nitric oxide synthase (NOS), and myeloperoxidase (MPO) (Saed et al. 2017). Cervical cancer, the fourth most common cancer among women, is generally caused by HPV infection (Bray et al. 2018). Oxidative stress is one of the crucial factors that foster the human papillomavirus (HPV) induced cervical cancer. The other cofactors that increase the risk of cervical cancer include smoking, oral contraceptives, and immunosuppression (Green et al. 2003). In general, cancer cells exploit ROS to maintain the oncogenic phenotype as ROS-mediated oxidative DNA damage leads to genomic instability, an evolving hallmark of cancer. Oxidative stress-induced genomic instability drives carcinogenesis by activating proto-oncogenes and downregulating the functioning of tumor suppressor genes (Sallmyr et al. 2008). The functioning of ROS is primarily dependent on its concentration as well as cellular localization. Novel therapies should aim at targeting intracellular ROS levels in cancer cells, to drive the ROS-associated cell survival towards ROS-associated cell death. Otherwise, the use of therapeutic antioxidants may help prevent early activities in tumor development, where ROS is essential (Trachootham et al. 2009). Even though the emphasis of this chapter is to uncover the role of ROS in tumor immunology from the therapeutic aspects, we cannot ignore the presence of reactive nitrogen species (RNS). Along with ROS, the production and signaling of RNS is known to be involved in the initiation, promotion, and progression of various cancers, including gynecological, gastric, breast, colorectal, and prostate cancer (Joanna and Hassan 2017). Here we aim to gather knowledge from published literature to make a cohesive graphical presentation of oxidative stress in ovarian and cervical cancer. Here we have emphasized on the underlying reasons for generation of oxidative stress due to imbalance between ROS production, and scavenging by antioxidants, the role of hence accumulated ROS in tumor cells, its effect on cell-cell communication and cellular immunology and also its modulation via therapeutics to treat cancers.

Nexus Between ROS, Cancer, and Immune Cells

Oxygen is an essential molecule for energy production and survival of an organism, but on the same hand, it can prove to be toxic under certain conditions. In a healthy cell, oxygen is utilized in the electron transport chain (ETC), where it acts as a final acceptor of electrons and gets reduced to water molecules. In this process, electrons may leak out from ETC complexes and reduce oxygen to produce superoxide radicles, one of the most common ROS. Superoxide radicle has low reactivity with biomolecules but it acts as a substrate for producing other potent reactive oxygen species such as hydrogen peroxide, a neutral species with high permeability across cell membranes. Hydrogen peroxide plays a role in cell signaling, and it is known that a small amount of it is required by healthy cells for their growth and proliferation. In healthy cells, the detoxification of superoxide radicles and hydrogen peroxide radicles by antioxidants keep their level below the threshold to avoid any adverse effect. Under conditions where the level of ROS rises above the threshold, oxidative stress is induced, here hydrogen peroxide reacts via Fenton reaction with

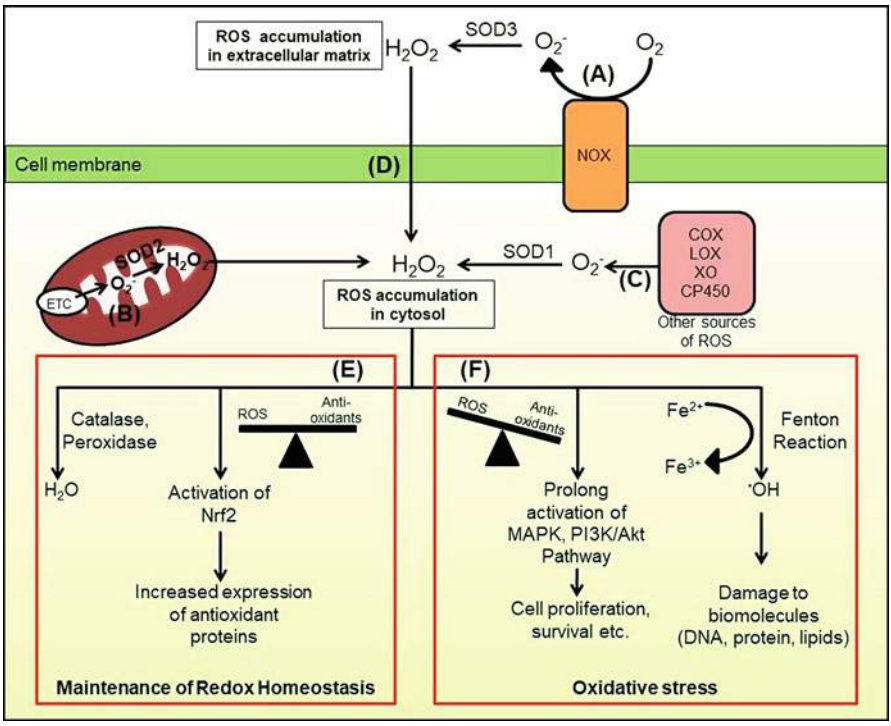

Fig. 1 Overview of redox homeostasis and oxidative stress in a cell. Reactive oxygen species are produced in extracellular matrix by (**a**) NADPH oxidase (NOX) and in cytosol by (**b**) mitochondrial respiration and (**c**) by the activity of other metabolic enzymes e.g. cyclooxygenase (COX), lipoxygenase (LOX), xanthine oxidase (XO) and cytochrome P450 (CP450). The superoxide radicles ($O^{-}{}_{2}{}^{\cdot}$) are converted to hydrogen peroxide (H_2O_2) by superoxide dismutase present in various subcellular locations i.e. cell cytosol, mitochondria and extracellular matrix. (**d**) H_2O_2 is permeable to membrane and accumulates in cell cytosol. (**e**) Generation of ROS activates Nrf2 transcription factor, which induces expression of antioxidant proteins. H_2O_2 is neutralized to water by catalase or peroxidase to maintain redox homeostasis. (**f**) Under stress conditions, H_2O_2 reacts with free iron to generate hydroxyl radicals (•OH). H_2O_2 if not neutralized causes aberrant activation of MAPK, PI3K/Akt pathways and •OH induces oxidative damage to cellular macromolecules (DNA, protein, lipids), inducing oxidative stress

free iron (Fe^{2+}) released under stress or with superoxide radical via Haber-Weiss reaction to produce hydroxyl radicals. Hydroxyl radical is the most potent cell-damaging species as well as a strong mutagen due to its strong reactivity with all the cellular biomolecules (Schieber and Navdeep 2014). Apart from mitochondrial respiration, the other sources of ROS are cytochrome P450 system of mitochondria which produces ROS during the metabolism of xenobiotics, NOX which produce superoxide radicles as their major catalytic product, cyclooxygenases (COXs), lipooxygenases (LOXs), xanthine oxidases (XOs) which produces ROS as one of their byproducts (Fig. 1) (Tafani et al. 2016).

Oxidative stress is one of the characteristic features of cancer cells that manipulate various pathways to increase the level of ROS. For instance, many human cancers are associated with defects in ETC complexes, producing a large amount of ROS (Raimondi et al. 2020). Cytochrome P450, another source of ROS, is overexpressed in ovarian and other cancers and is associated with chemotherapeutic resistance (Androutsopoulos et al. 2013; Zhu et al. 2015). Various NOX isoforms, for example, NOX1–4 are found to be overexpressed in ovarian cancer, colon cancer, breast cancer, prostate cancer, etc. (Juhasz et al. 2009; Meitzler et al. 2017).

Other than the abovementioned sources of ROS, a constant source for extracellular ROS generation is the tumor-associated inflammatory cells present in the tumor microenvironment (TME). These cells play an essential role in cancer initiation and progression by mediating tumor growth, invasion, angiogenesis, and metastasis, and they also suppress antitumor immune responses. These cells mainly include TAMs and MDSCs. TAMs are the pro-tumor macrophages that originate from blood monocytes that infiltrate into the TME where they get polarized to tumor-promoting macrophages from antitumor cell type. MDSCs are a heterogeneous group of immature myeloid cells with intense immunosuppressive activity. The depletion of TAMs and MDSCs in preclinical tumor models inhibits tumor progression accompanied by increased T cell activity. The immunosuppressive function of both TAMs and MDSCs is dependent on ROS and administration of ROS inhibitors abolished the immunosuppressive activity of both cell types in vitro (Ugel et al. 2015; Zhang et al. 2013).

Functions of ROS in healthy cells or cancer cells depend on its concentration as well as on the length of exposure. At low or moderate levels, ROS acts as a signaling molecule and stimulates cell growth, whereas at higher levels, it can cause severe damage to biomolecules leading to cell death (Schieber and Navdeep 2014). Cancer cells persistently maintain higher ROS levels than healthy cells but below the threshold at which ROS shows its cytotoxic effects. The levels of ROS are maintained by various antioxidants including enzymatic antioxidants namely superoxide dismutase (SOD), catalase, and glutathione (GSH)-thioredoxin (Trx) system enzymes as well as nonenzymatic antioxidants such as glutathione, vitamin C, vitamin E, carotenoids, etc. (Joanna and Hassan 2017). SOD is a metalloenzyme that acts as a primary defense system against superoxide radicals. Cu/Zn SOD (SOD1) is present in cytosol, Mn-SOD (SOD2) in mitochondria, and another Cu/Zn-containing SOD (SOD3) is present in the extracellular matrix. SOD catalyzes the dismutation of superoxide radicals into molecular oxygen O_2 and hydrogen peroxide. Hydrogen peroxide thus produced is reduced to water by the enzyme catalase or glutathione peroxidase (GPx) (Fig. 1). Nuclear factor erythroid 2–related factor 2 (Nrf2) is a crucial transcription factor that regulates the expression of these antioxidant enzymes and various other antioxidants involved in detoxification of ROS. The activity of Nrf2 is controlled by a redox sensor protein named Kelch-like ECH-associated protein 1 (Keap1), which forms a complex with cul3 and Rbx1 forming an active E3 ubiquitin ligase. This complex binds to Nrf2 in the cytosol, inhibits its translocation to the nucleus, and mediates proteasomal degradation. Under oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus where it binds to

antioxidant response elements (ARE) and activates the transcription of antioxidant genes (Soraya and Mozafar 2018). Nrf2, a master regulator of the antioxidant defense system, is one of the prime targets that cancer cells hijack. In ovarian cancer, aberrant activation of Nrf2 has been observed, promoting the growth of tumor cells in a highly oxidative environment. Other studies also reveal that most of the Nrf2 aberrant activity might be attributed to the inactivating DNA alterations (inactivating mutations, copy number loss, hypermethylation) in any of the components of the E3 ubiquitin ligase complex *KEAP1–CUL3–RBX1* (Martinez et al. 2014). The resulting constitutive activation of Nrf2 corresponded to increased expression of Gpx3 and SOD2 (Konstantinopoulos et al. 2011). A similar trend has been observed in cervical cancer patients as well (Ma et al. 2015). In addition to supporting the growth of tumors in an oxidative environment, Nrf2 overactivation provides chemoresistance against drugs that are known to increase the ROS levels (such as platinum-based drugs) in cancer cells by inducing cell cytotoxicity (Konstantinopoulos et al. 2011).

The interplay between redox homeostasis, tumor cells, and immune cells paves the way for the progression of cancer through all its stages. Cancer cells exploit various strategies to survive and reproduce, such as increasing intracellular as well as extracellular ROS production while regulating the activity of antioxidants to surpass the ROS-induced cell-cytotoxicity. In the successive sections, we are going to explore the exact mechanism by which healthy tissues undergo a neoplastic transformation and how they utilize this redox biology and immune cells to expand exponentially in ovarian and cervical cancer, the two most fatal gynecological cancers.

Chronic Inflammation-Related Oxidative Stress in the Pathogenesis of Ovarian and Cervical Cancers

The process of carcinogenesis is divided into three phases, which involve initiation, promotion, and progression. In the initiation phase, healthy cells undergo irreversible genetic mutations which may be induced due to extrinsic factors like chemicals, radiations, heavy metals, viruses, or, intrinsic factors, including chronic inflammation, defective DNA repair pathways leading to dysregulation of cell signaling pathways related to cell proliferation, survival, etc. The second phase of cancer development is long and reversible and involves a selective clonal expansion of initiated cells by sustained cell growth signaling, inhibiting cell apoptosis, and escaping immune surveillance. In the final, irreversible progression phase, cells acquire malignant phenotype and become invasive (Fig. 2). In all the three phases of carcinogenesis, oxidative stress induced by tumor cells as well as by tumor-associated immune cells plays pivotal roles which we will be dissecting one by one in the following sections.

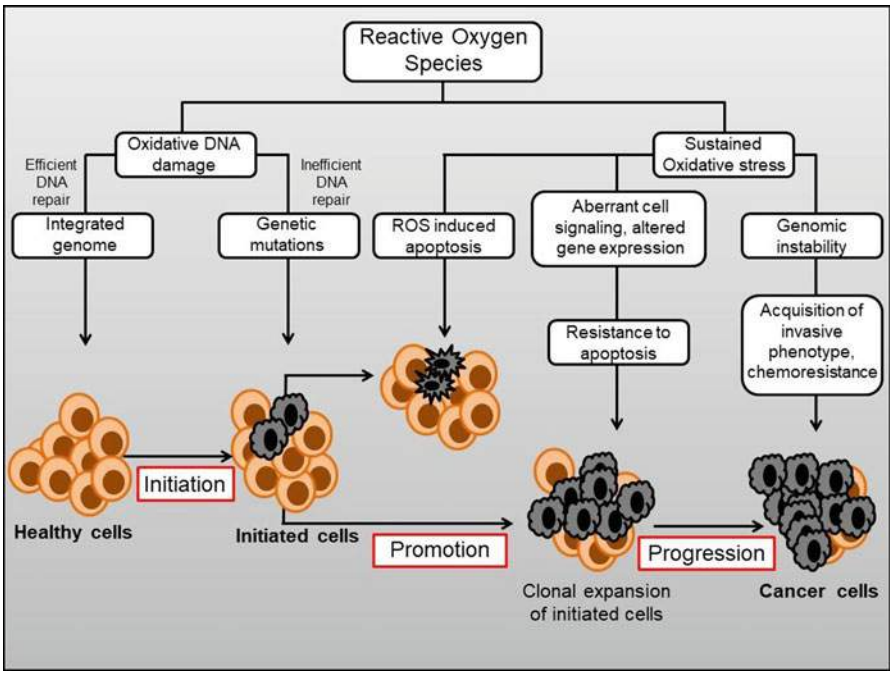

Fig. 2 Role of reactive oxygen species in carcinogenesis. Oxidative stress in healthy cells induces DNA damage which if not repaired by DNA repair machinery leads to genetic mutations (initiation phase). Persistent oxidative stress can lead to apoptosis or activation of proto-oncogenes and inactivation of tumor suppressor genes in the initiated cells. The initiated cells lose the ability to control cell cycle and gain resistance to apoptosis which leads to clonal expansion (promotion phase). In progression phase, sustained oxidative stress leads to genomic instability and cells acquire invasive phenotypes (ability to undergo epithelial to mesenchymal transition) and become malignant

Oxidative Stress in Ovarian and Cervical Cancer Initiation

In this section, we are going to discuss oxidative stress in the context of imbalance between free radicals, antioxidants, and immune responses in ovarian and cervical cancer. Tissues which are exposed to continuous inflammatory stress and are actively infiltrated with immune cells due to infection or various other reasons are known to undergo neoplastic transformation. This indicates that interplay between inflammatory immune cells and neoplastic cells is essential for the development and progression of various cancers. Inflammatory cells produce various growth factors, cytokines, as well as reactive species, thus inducing localized oxidative stress. Persistent inflammation due to repeated tissue damage or infection exposes tissue to oxidative stress leading to DNA damages in proliferating cells, resulting in tissue damage or cell transformation (Coussens and Werb 2002). In context to this, ovary and cervix tissue are the appropriate examples to give, as ovaries are under repeated cycles of wounding and repairing during each menstrual cycle, and is believed to be

one of the causes of ovarian cancer. In contrast, cervical cancer has been observed to be caused by persistent HPV infection. Both of these scenarios are associated with chronic inflammation-induced oxidative stress and cancer initiation.

There are mainly two hypotheses that support the role of oxidative stress in ovarian cancer initiation. One is "incessant ovulation" theory and the other one is "incessant menstruation" theory, both of which propose that oxidative stress-induced during the ovulatory cycle and retrograde menstruation is one of the primary cause of ovarian cancer (Hakim et al. 2009; Vercellini et al. 2011; White et al. 2014).

Females undergo repetitive cycles of ovulation, which begins at puberty, known as menarche and ceases around the age of 50–60 years, known as menopause. ROS is essential at each phase of the menstrual cycle, namely the follicular phase, ovulation phase, and luteal phase. During the follicular phase, the level of cellular ROS rises with the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). LH induces the expression of nitric oxide synthase III (NOS III), the enzyme that catalyzes the formation of nitric oxide (NO). NO is essential for the growth of follicles and ovulation. The levels of ROS are maintained by various antioxidants (mainly SODs) during the cycle to maintain ROS homeostasis. The release of the oocyte into the fallopian tube (ovulation) is associated with the rupturing of ovarian surface epithelium (OSE), which then proliferates back to repair the surface. Disruption of OSE leads to the infiltration of leukocytes (as a part of the wound healing process), which causes inflammation by releasing various cytokines, NO, and ROS, thus adding to the oxidative stress. After ovulation, the ruptured follicle transforms into the corpus luteum. In the absence of fertilization, corpus luteum degenerates, and this is associated with leukocyte infiltration, further adding to ROS levels. In conclusion, the repetitive proliferation of OSE and inflammation-induced oxidative stress during each ovulatory cycle accumulate mutations in the DNA (genetic instability) and can cause cellular transformation – incessant ovulation hypothesis (White et al. 2014). This hypothesis is supported by the observation that the incidence of p53 mutations is found to be more in ovarian cancer patients with a higher number of ovulatory cycles (Hakim et al. 2009).

The incessant menstruation hypothesis states that oxidative stress is induced by free iron released due to the hemolysis of menstrual blood. Because of the retrograde menstruation (reflux of menstrual blood into the pelvis), menstrual blood containing endometrial cells enters into the pelvis, which activates the pelvic macrophages resulting in an inflammatory response and lysis of red blood cells (hemolysis). Thus, released free iron act as a catalyst and generates ROS via Fenton reaction thus inducing oxidative stress. The fimbriae of fallopian tubes in peritoneum get exposed to the bloody peritoneal fluid with free iron-induced oxidative stress which over time may cause cellular malignancy (Vercellini et al. 2011).

Oxidative stress also has implications in cervical cancer initiation. Cervical cancer is associated with Human papillomavirus (HPV) infection. HPVs are non-enveloped viruses with circular double-stranded DNA. Two high-risk HPV types – HPV16 and HPV18 – have been detected in the majority of cervical cancer cases (Chen Wongworawat et al. 2016). HPV-mediated carcinogenesis involves the integration of its genome into the host genome resulting in overexpression of two viral

proteins – E6 and E7. E6 protein binds to tumor suppressor protein p53 resulting in ubiquitinylation and proteasome-mediated degradation of p53 whereas E7 binds to hypo-phosphorylated retinoblastoma protein and inhibits its binding to E2F transcription factor resulting in dysregulation of cell cycle leading to cellular transformation. The integration of the HPV genome into the host genome is essential for cellular transformation. This genomic inclusion may be controlled by oxidative stress and inflammation. The viral DNA is replicated to sufficient levels so that the virus and its proteins can be detected by immune cells at later stages of HPV infection. The HPV-mediated carcinogenesis depends on several cofactors, including smoking (known inducer of inflammation) and coinfection with a sexually transmitted disease, either virus or bacteria. Infection with herpes simplex virus (HSV) or with *Chlamydia trachomatis* (bacteria) induces a strong inflammatory response by recruiting leukocytes, which release a lot of inflammatory cytokines as a defense mechanism against pathogens. Such coinfection-induced inflammation increases ROS production by innate and adaptive immune cells and induces the formation of DNA double-strand breaks which is essential for HPV genome integration and hence carcinogenesis (Chen Wongworawat et al. 2016; De Marco 2013; Williams et al. 2010). Concerning this, it was seen that depletion of intracellular antioxidant GSH increases the incorporation of the HPV16 genome into the host genome of cervical keratinocytes, and reversing the condition dramatically reduces the rate of viral genome integration (Chen Wongworawat et al. 2016).

Oxidative Stress in Ovarian and Cervical Cancer Promotion

Cancer cells persistently maintain a high level of ROS as compared to their healthy counterparts. The continuous presence of ROS is required by tumor cells for their survival, proliferation, and to maintain genomic instability, angiogenesis, as well as in escaping immune surveillance, all of which are hallmarks of cancer. These effects of ROS can be attributed to its ability to cause DNA damage, to induce genetic mutations. These effects may, in turn, alter the expression of genes (overexpression of oncogenes or downregulation of tumor suppressor genes) and activate various cell signaling pathways to modulate tumor microenvironment (Tafani et al. 2016). Ovarian cancer cells have overexpression of NOX4, which produces superoxide radicals as its main product, thus acting as a continuous source of ROS (Juhasz et al. 2009). The superoxide radicals hence generated, by the activity of SOD, undergo dismutation to form hydrogen peroxide, a signaling molecule, and modulator of various pathways.

Oxidative Stress in Cell Proliferation and Survival

Cell growth and proliferation depend on the availability of growth factors that stimulate mitogenic pathways through the activation of receptor tyrosine kinases (RTKs). RTKs further activate phosphatidylinositol 3-kinase (*PI3K*)/protein kinase B (*AKT*) and *Ras*/Raf/mitogen-activated protein kinase (MAPK)/*ERK* kinase (*MEK*)/extracellular signal-regulated kinase (*ERK*) pathway through sequential

phosphorylation of downstream proteins to stimulate cell growth and proliferation. Protein tyrosine phosphatase (PTP) 1B, mitogen-activated protein kinase phosphatases (MKPs), and phosphatase and tensin homolog (PTEN) phosphatase dephosphorylate and inhibits RTKs, ERK1/2, and PI3K activity respectively, thus impeding cell proliferation. Growth factors mediate their effect by activating NOX enzyme, transiently increasing the production of ROS, which in turn inhibits the phosphatase activity, hence promoting cell proliferation and growth. Sustained high ROS levels inactivate PTP1B, PTEN, and MKP, enhancing cell proliferation. ROS, specifically hydrogen peroxide, does so by oxidizing the thiol group of cysteine residue required for the catalytic activity of PTP1B and PTEN, resulting in inhibition of phosphatase activity (Schieber and Navdeep 2014). Akt enzyme supports the cell growth and survival in two ways which include phosphorylation-dependent inactivation of pro-apoptotic proteins and activation of pro-survival proteins. For example, Akt phosphorylates and inhibits the function of apoptosis promoting proteins, namely, Bad (BCL2 associated agonist of cell death) and caspase-9 (an initiator of apoptotic pathway), as well as, stimulates the activity of transcription factors, such as, nuclear factor-κB, which induces the expression of pro-survival genes. ROS accumulation in ovarian cancer cells may induce proteasome-mediated degradation of MKP3 (an inhibitor of ERK1/2), activating the MAPK pathway, promoting tumorigenesis (Chan et al. 2008).

Oxidative Stress in Angiogenesis

The formation of new blood vessels from preexisting capillaries is referred to as angiogenesis. Due to excessive tumor proliferation, the size of the tumor, as well as the distance between blood vessels and cancer cells, get increased, causing hypoxia. Angiogenesis is required to supply the oxygen and nutrients to cancer cells and thus needed for tumor growth, survival, and metastasis. ROS directly affects the process of angiogenesis by stabilizing hypoxia-inducible factor-1α (HIF-1α) by inactivating its inhibitor, prolyl hydroxylase. HIF-1α then dimerizes with HIF-1β to bind and activate the expression of several genes, including vascular endothelial growth factor (VEGF) and its receptors (Chandel et al. 2000). In ovarian cancer cells, hydrogen peroxide is responsible for HIF-1α stabilization and the production of VEGF for neovascularization during hypoxia. Inhibition of NOX4 or overexpression of catalase inhibits the HIF-1α stabilization and VEGF production, thus angiogenesis (Xia et al. 2007).

Oxidative Stress in Immune Suppression

The immune cells continuously monitor the host's system to protect it from foreign invaders. Although cancer cells arise from the host's healthy cells, they do differ from their regular counterparts. Thus, an immune cell scan recognizes cancer cells, generating an immune response. Cancer cells evade the immune attack by various means which include secretion of immunosuppressive cytokines, downregulation of immunogenic antigens from the cell surface, acquiring resistance to death effector mechanisms of cytotoxic immune cells, etc. Cancer cells utilize a combination of these strategies to combat immune attack (Urszula and Krzysztof 2016). But one of the vital

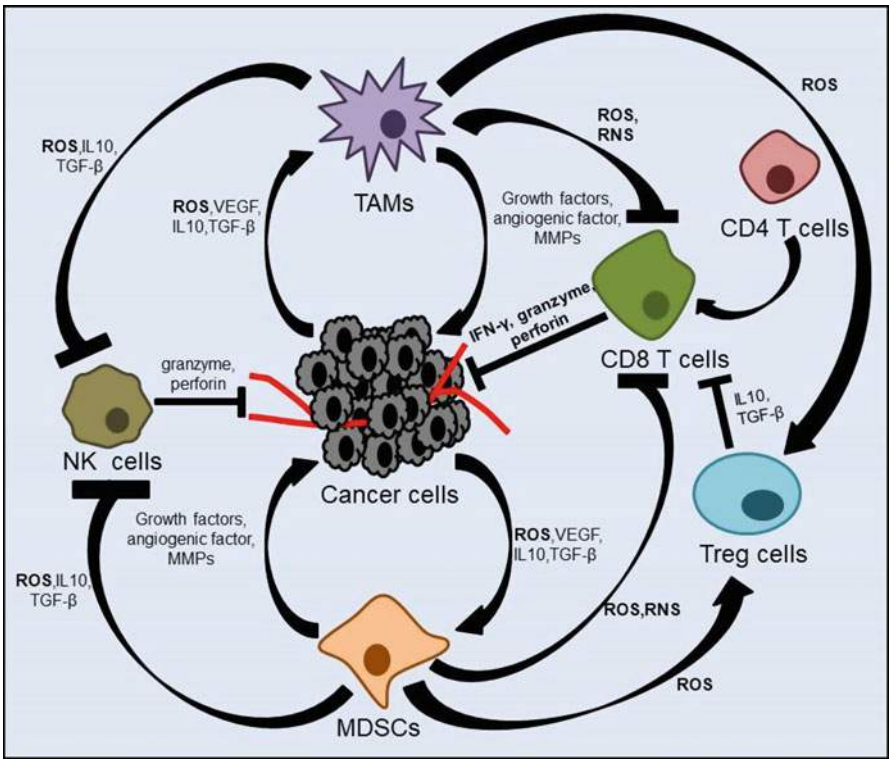

Fig. 3 ROS induced immunosuppression in tumor microenvironment. ROS released from cancer cells is required for polarization of pro-inflammatory macrophages to anti- inflammatory cell type as well as for induction of myeloid derived suppressor cells (MDSCs). Tumor associated macrophages (TAMs) and MDSCs releases various growth factors, pro-angiogenic factors to support the growth of cancer cells. They also secret immunosuppressive cytokines as well as ROS to inhibit the activation and function of CD8 T cells and NK cells, the essential part of antitumor immunity. ROS derived from TAMs and MDSCs also contributes to induction of regulatory T cells (Tregs) thus maintaining the immunosuppressive tumor microenvironment

strategies that cancer cells use is the polarization of antitumor innate immune cells (myeloid cells) to pro-tumor cell type by secreting cytokines like interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), VEGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), etc. These pro-tumor cells, which are known as TAMs and MDSCs, are highly immunosuppressive as they directly or indirectly suppress T cell activity via ROS production (Fig. 3) (Ugel et al. 2015). ROS production in TAMs and MDSCs is mediated majorly by NOX2 isoform, also known as phagocytic NOX. ROS derived from these cells downregulates the expression of T-cell co-receptor (specifically CD3ζ chain) expression and cytokine secretion, thus inhibiting T cell activation (Schmielau et al. 2001). MDSCs inhibit the interaction between MHC (I)-peptide complex and TCR-CD8 receptor of T cells via producing ROS and peroxynitrite (RNS), which modifies the structure of TCR and CD8

molecule. T cell loses the ability to recognize MHC I-peptide complex, inducing T cell tolerance against specific antigen (Nagaraj et al. 2007).

Furthermore, RNS released from suppressor cells modify chemokine (CCL2, CCL5) structure by nitration and nitrosylation, inhibiting the recruitment of lymphocytes to the tumor site (Molon et al. 2011). ROS production by macrophages can also stimulate the induction of regulatory T cells (Tregs), and this ability of macrophages is halted by inhibition of ROS generation. The presence of TAMs, MDSCs, and Tregs found in ovarian and cervical cancer patients causes poor prognosis and survival (Kraaij et al. 2010).

Oxidative Stress in Ovarian and Cervical Cancer Progression

The increased oxidative stress in the tumor microenvironment helps in acquiring a more invasive phenotype via stimulating epithelial to mesenchymal transition (EMT) and chemotherapeutic resistance. Tumor cell migration and metastasis involve downregulation of cell-cell junctional proteins, loss of cell-matrix interacting proteins, surpassing anoikis (anchorage-dependent cell death), breaching basement membrane, as a part of ROS activity (Jiang et al. 2017). ROS stabilizes the HIF-1α to initiate angiogenesis; the other important role of HIF-1α is the downregulation of E-cadherins (a feature of EMT) through overexpression of lysyl oxidase in ovarian cancer cells, though the exact mechanism is still unclear (Wang et al. 2014). Many growth factors that are involved in tumor cell metastasis induce NOX, and ETC mediated ROS generation. ROS, specifically hydrogen peroxide, in turn, activates various kinases via MAPK, PKC, PI3K/Akt, and FAK (focal adhesion kinase) all of which regulate cell mobility (Amer and Oliver 2012). One of the critical roles in facilitating tumor cell invasion is of matrix metalloproteinases (MMPs), enzymes that degrade extracellular matrix proteins. MMPs are overexpressed in various cancer types, including ovarian and cervical cancer. High levels of MMP2 and MMP9 are associated with malignant ovarian and cervical cancer (Amer and Oliver 2012). MMPs expression either directly or indirectly increases ROS generation. For instance, binding of integrins to their respective ligand activates a small GTPase known as Rac1, which stimulates NOX, 5-lipoxygenase, and mitochondria to release ROS. ROS inhibits various phosphates and thus activates the MAPK pathway, which induces the expression of different MMPs depending on the type of stimulus (Svineng et al. 2008).

Modulation of ROS as Cancer Therapy

Chemotherapy

The first line of treatment used for cervical and ovarian cancer patients is cytoreductive surgery followed by the administration of radio-chemotherapy using platinum-based drugs such as cisplatin, carboplatin, etc. and taxanes. Over the years,

nearly 80% of the patients provided with this therapy tend to relapse within 2 years, with acquired resistance to the platinum-based drugs, which is most likely incurable. Cancer stem cells with help from ROS are suspected to be the reason behind the newly acquired resistance of cancer cells to chemotherapy (Foster et al. 2013; Garson and Vanderhyden 2015).

Doxorubicin

One of the widely used cancer drugs is doxorubicin, derived from bacteria of genus *Streptomyces*. Doxorubicin causes cancer cell growth inhibition via blocking the topoisomerase-II mediated DNA repair by intercalating into the DNA strand. Doxorubicin shuttles between its oxidative form semiquinone, that when converts back to doxorubicin liberate reactive oxygen species, more specifically superoxides (Thorn et al. 2011). Studies suggest that the differential sensitivity of cancer cells to this drug is associated with modulation in levels of ROS related protein expression. SiHa, a human cervical cancer cell line that is more resistant to doxorubicin than CaSki showed an evident increase in expression of NAD(P)H quinone dehydrogenase 1 (NQO1), peroxiredoxin 2 (PRDX2), SOD1, SOD2, Gpx1/2, cytochrome b-245, alpha polypeptide (CYBA), which lead to a reduction in the level of free radical in cells. ROS producing proteins such as aldehyde oxidase 1 (AOX1), NADPH oxidase complex (NCF2), and oxidation resistance protein (OXR1) are downregulated in SiHa when compared with CaSki (Filippova et al. 2014). The interplay of different pro- and antioxidant enzyme levels in cancer cells suggest the presence of varied baseline ROS expression, which contributes to the differential sensitivity of these cells to doxorubicin. Hence, the higher the baseline level of ROS in a cancer cell, the more susceptible it will be to chemotherapeutics such as doxorubicin that work by further increasing ROS levels in the cells. The increase in superoxides following treatment of cells with doxorubicin has been thought to be associated with the inactivation of enzymes SOD2 and catalase that decompose superoxides.

Platinum-Based Drugs (Cisplatin, Carboplatin)

Cisplatin is being used as the conventional mode of treatment for ovarian and cervical cancers currently, though useful only when used as the first line of treatment. Platinum-based drugs use copper transporters to enter into mammalian cells; once inside a cell, their chloride group is replaced by a water molecule and they can attack purines in DNA. These drugs bind to DNA, and inter-strand cross-linking of purines takes place, making DNA unavailable for protein binding (Dasari and Tchounwou 2014). It has been found that apart from the effect cisplatin has on the DNA of a cell, it is also involved in elevating the ROS level in cancerous cells. The specific reactive oxygen species whose production is increased are hydrogen peroxide, hydroxyl, and peroxyl radicals. Cisplatin-treated cells show decreased levels of peroxynitrite which explains the increased presence of hydroxyl radical (Beckman et al. 1990). Once inside cell, cisplatin tends to bind GSH through sulfhydryl groups, hence making it unavailable to carry out its antioxidant properties, and leading to an increase in levels of hydrogen peroxide and hydroxyl radicals (Luanpitpong et al. 2012).

Another study suggests that when ovarian cancer patients with estrogen-dependent tissues are exposed to carboplatin, the patients sensitive to the drug showed

higher initial levels of antioxidant proteins and uncoupling proteins compared to patients resistant to treatment. Treatment-induced oxidative stress causes inhibition of tumor growth in disease-sensitive patients as oppose to the reduced amount of generation of oxidative stress in resistant patients, due to low initial levels of the antioxidant response, which helps cells escape stress caused by carboplatin treatment (Pons et al. 2012).

Studies suggest that ROS plays a dual role in chemotherapy; it is involved in chemosensitivity and chemoresistance of cisplatin both in vivo and in vitro. The profiling of ROS pathway genes leads to the segregation of ovarian cancer patients into the low-score and high-score patients through which prognosis of the disease and response to platinum-based and taxane-based drugs in these patients can be predicted beforehand. This scoring system has high clinical significance as prior knowledge of the ROS gene profile would help select a suitable chemotherapeutic drug for effective treatment and avoid unnecessary toxicity caused by the use of medications that the patients may be resistant to (Sun et al. 2019).

Paclitaxel

Paclitaxel targets microtubules to carry out anticancer therapy. A cell needs to have dynamic microtubule polymerization for its functioning, which is stabilized by the administration of paclitaxel. Thus, stabilized microtubule polymerization leads to failure of the cell to carry out many cellular processes like mitosis, transport of protein, organelle, and movement (Weaver 2014). Administration of paclitaxel leads to an extracellular release of ROS in the medium inducing apoptosis in adjacent cells (Alexandre et al. 2007; Panis et al. 2012).

Poly (ADP-Ribose) Polymerase (PARP) Inhibitors

Various clinical trials noted the effect of PARP inhibitors (PARPi) on ovarian cancers. These inhibitors majorly target PARP1 protein, which is involved in sensing and repairing breaks in the DNA. Recent studies suggest that PARP1 also plays a role in generating a cellular response to oxidative DNA damage (Ray Chaudhuri and Nussenzweig 2017). PARPi such as PJ-34, niraparib, and olaparib is used to study the effects they have on ovarian cancer in vivo and in vitro. Studies suggest that PARP hyperactivation induces ROS formation, which helps ovarian cancer cells to proliferate, interestingly administration of PARPi leads to a further elevation in ROS levels, which is evident by reduction in free glutathione/oxidized glutathione (GSH/GSSG) ratio and increase in superoxide levels.

Furthermore, the use of N-acetyl cysteine (NAC), a replenisher of GSH, resulted in rescued tumor growth and reduced level of DNA double-strand breaks, whereas the administration of hydrogen peroxide with PARPi leads to further death of tumor cells. NOX1 and NOX4 get upregulated in response to PARPi, the use of setanaxib/GKT, an inhibitor of NOX1 and NOX4 attenuates elevation of ROS in response to PARPi both in vitro and in vivo. The depletion of NOX has been associated with reduced DNA damage and hence rescued growth inhibition. These findings suggest that the administration of PARPi leads to the upregulation of NOX1 and NOX4; thus, ROS is induced, which leads to inhibition of cancer cell growth via oxidative DNA damage (Hou et al. 2018).

Immunotherapy

Immunotherapy involves the modulation of patients' own immune system to increase the cytotoxic efficiency of immune cells against cancerous cells. The use of immune check point inhibitors, cancer vaccines, antitumor monoclonal antibodies, cytokines, and adoptive T-cell therapy are the various immunotherapeutic strategies clinically accepted for cancer treatment. Among these approaches, adoptive T-cell therapy, cancer vaccines, and checkpoint inhibition therapy are the most widely accepted approaches for cancer immunotherapy (Byun et al. 2017; Hu et al. 2018; Restifo et al. 2012), due to effective cost and easy accessibility. Adoptive T-cell therapy involves the transplantation of T-cells into the host after being expanded ex vivo. Despite adoptive T-cell therapy is a novel antitumor therapy, it has its limitations, one of which is the inability of T-cells to proliferate and function in the tumor microenvironment due to the presence of high amounts of ROS. The efficiency of these techniques is needed to be advanced further by exploiting the interplay of ROS molecules with immune cells in play. The use of T cells with tumor-specific chimeric antigen receptor co-expressing catalase for adoptive T-cell therapy has proved to bring down the intrinsic as well as extrinsic reactive oxygen species in TME enabling T-cell capable of proliferation and carrying out antitumor functions (Ligtenberg et al. 2016).

In corroboration with the capacity to carry out antitumor functions, the engineered T-cells with catalase activity protect tumor-infiltrating T-cells and natural-killer (NK) cells from inactivation as part of their bystander effect (Ligtenberg et al. 2016).

Another exciting finding suggests that the use of chemotherapeutic agent Cytoxan (CTX) along with CD4+ T-cells adoptive immunotherapy leads to deficiency in the generation of GSH as a result of the destruction of multiple metabolic pathways of tumor cells thus increasing level of ROS in the TME. Hence accumulated ROS leads to decay of vessel-intensive tumors (rich in blood vessels) by rendering biomolecules such as DNA, proteins, and lipids of the tumor cells instable (Habtetsion et al. 2018). Thus, both accumulation and reduction of ROS at the TME may help treat a tumor in association with adoptive T-cell immunotherapy.

Conclusion

The triad of reactive oxygen species, immune cells, and healthy cells is crucial for necessary alterations for the transformation of cells to neoplastic cells contributing to different types of cancers. Gynecological cancers impose a huge burden on women's health, therefore on the nation. The involvement of ROS in gynecological cancers as well as in other cancers and diseases makes it essential for us to get a deep understanding of its various cellular functions and regulation. Although ROS helps in providing immunity against various pathogens, cancer cells use ROS as armor to protect themselves from immune attack by maintaining an immunosuppressive microenvironment. Several studies suggest that the use of chemotherapeutics that

target reactive oxygen species directly for cancer treatment has huge potential. The use of drugs that lead to the accumulation of ROS as well as the ones that have antioxidant properties, both have a degradative effect on cancer cells. It has also been observed that despite immunotherapy being a reliable method for cancer treatment, it has its limitations. Immunotherapy can be made more effective by (1) administrating it along with drugs that lead to ROS accumulation such that apoptotic pathways of the neoplastic cells get activated or (2) administrating immunotherapy along with antioxidants such that ROS is made scarce in the tumor microenvironment, hence being unable to inactivate immune response such as T-cell proliferation and anti-tumor functioning. A better understanding of this field is needed so that the ROS axis can be better exploited to treat various cancers.

Cross-References

- ▶ Biomarkers of Oxidative Stress and its Dynamics in Cancer
- ▶ DNA Lesions Induced by Lipid Peroxidation Products in Cancer Progression
- ▶ Environmental Contaminants, Oxidative Stress and Reproductive Cancer
- ▶ Reactive Oxygen Species and Their Contribution to Carcinogenesis
- ▶ Relationship Between Oxidative Stress and Immune Response in Cancer
- ▶ Ros in Apoptosis of Cancer Cells
- ▶ The Implication of Ros Homeostasis in the Modulation of Emt Signaling and its Role in Manipulating Tumor Microenvironment
- ▶ Understanding ROS Induced DNA Damage for Therapeutics

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In-vitro Cervical Cancer model to study the efficacy of Radiomimetic in combination with Platinum-based drugs

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Introduction

Cancer is the second leading cause of death globally, and is responsible for an estimate of 9.6 million deaths in 2018. Globally, about 1 in 6 deaths happen due to cancer. It has effected India in a major way by causing 0.75 million deaths in 2018. Cervical cancer is the second most common cancer in Indian women accounting for 22.86% of all cancer cases in women.

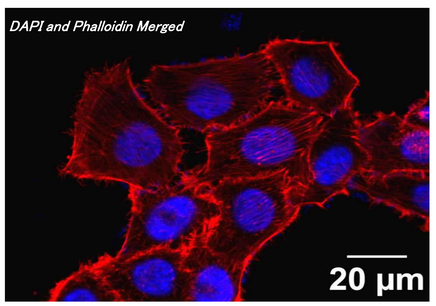
According to the American Society of Cancer, high priority drugs used as cancer therapeutic are platinum-based. In India, lack of awareness and instrumentation leads to late diagnosis of cancer, compelling administration of higher concentration of platinum-based drug as therapeutics. The side-effect of using these drugs as a mode of treatment is development of Nephrotoxicity in large population of women, hence to overcome this situation, the use of another drug that would sensitize cancer cells towards platinum-based drugs would help reduce the effective concentration of it being used for treatment in patients.

Combinational therapies are in use to increase the efficacy of the drugs as well as to reduce their side effects. Here we are using Bleomycin for our pilot experiments. Bleomycin is a glycopeptide antibiotic with a unique mechanism of antitumor activity. It induces DNA single-strand and double strand breaks via binding to DNA and also by mediating ROS production. As the outcome of using bleomycin is similar to that of radiations, we have used it as a radio mimetic agent for our studies.

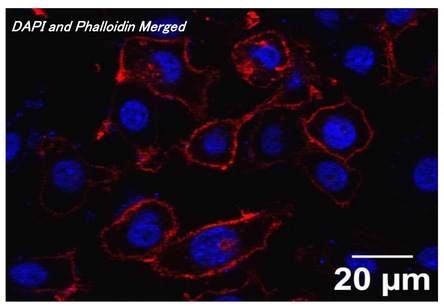
Methods

- Phalloidin staining for morphological characterization of Human cervical cancer cell lines namely ME-180, HeLa, SiHa, C33a.
- Bleomycin is used as a radiomimetic drug.
- MTT Assay-Cell viability assay to calculate the IC50 for each cell line.
- Propidium iodide staining- Cell cycle analysis to assess the effect of bleomycin on cell cycle .

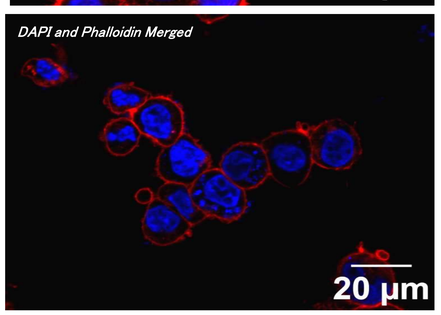
Results



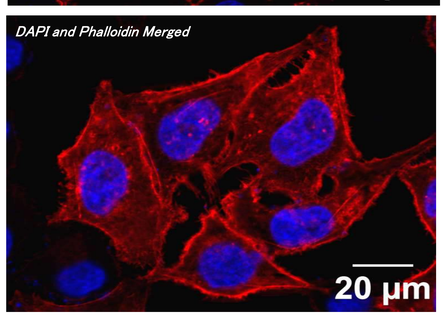
SiHa cells (ATCC HTB-35) Human Cervical Epithelial Cell lines Polygonal shape



ME-180 cells (ATCC HTB-33) Human Cervical Epithelial Cell lines Polygonal shape



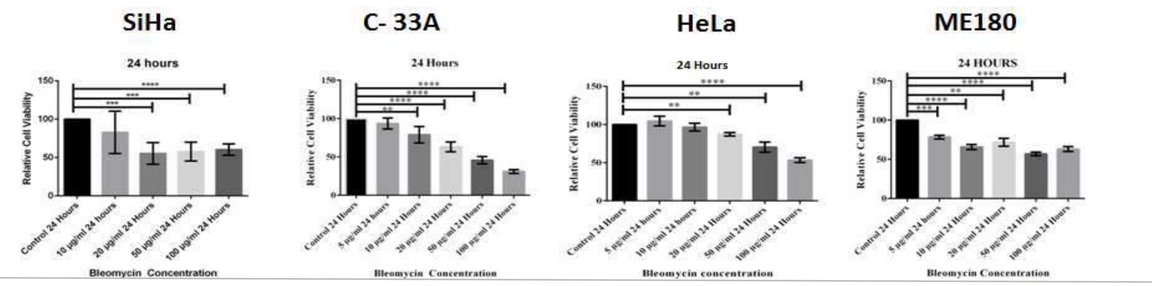
C33a cells (ATCC HTB-31) Human Cervical Epithelial Cell lines Round shape

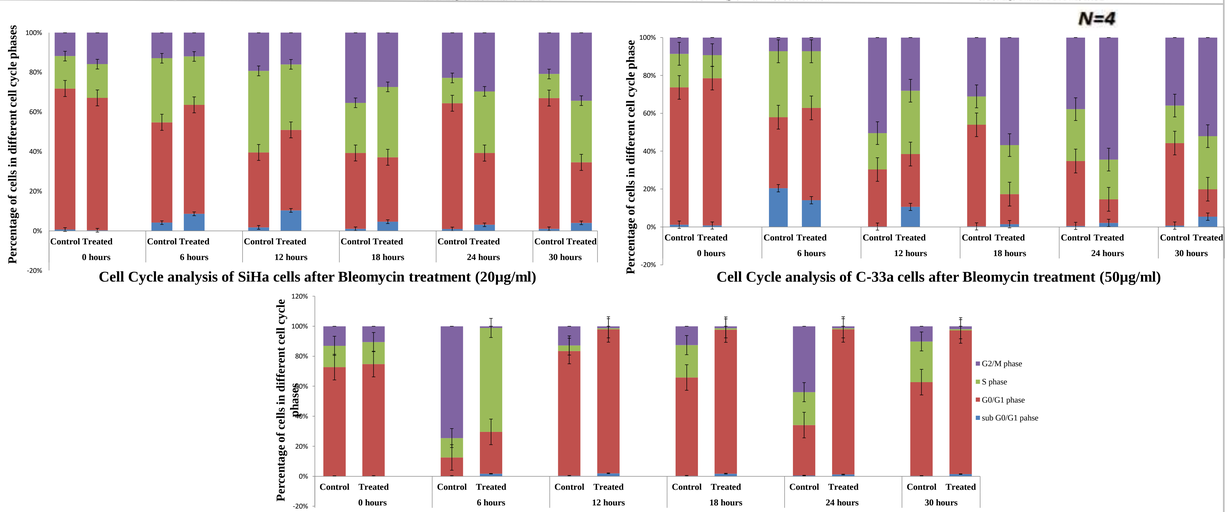


HeLa cells (ATCC CCL-2) Human Cervical Epithelial Cell lines Polygonal shape

Differential sensitivity of cells to Bleomycin treatment

	SiHa	>	C- 33A	>	HeLa	>	ME180
Observed IC50 values	20µg/ml		50µg/ml		100µg/ml		>100µg/ml

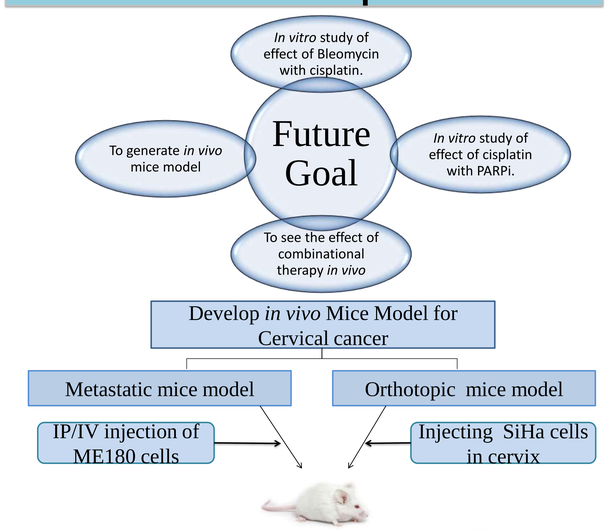




Conclusion

1. All cell lines respond to bleomycin treatment in a dose dependent manner
2. All cell lines are differentialy sensitive to Bleomycin – SiHa being most sensitive and ME-180 being most resistant.
3. The G2/M phase and S phase of cell cycle in C33a and SiHa cells gets elongated.
4. HeLa cells get arrested at G0/G1 phase of cell cycle.

Future Perspective



Development of Cervical Cancer models to study efficacy of Various Chemotherapeutics

Acknowledgement

1. This work has been supported by the System Medicine Cluster (SyMeC), **Department of Biotechnology**, India.
2. I want to acknowledge IISER-K for fellowship.
3. All my labmates and my supervisor Prof. Jayasri Das Sarma.

Reference

1. Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. J Natl Cancer Inst. 2013;105(7):452-8.
2. Prasad CB, Prasad SB, Yadav SS, et al. Olaparib modulates DNA repair efficiency, sensitizes cervical cancer cells to cisplatin and exhibits anti-metastatic property. Sci Rep. 2017;7(1):12876. doi:10.1038/s41598-017-13232-3
3. Tang, M., Liu, Q., Zhou, L. et al. Invest New Drugs (2018). doi.org/10.1007/s10637-018-0616-7

A pilot experiment to characterize Cervical cancer cell lines and study the effect of radio-mimetic drug, Bleomycin.

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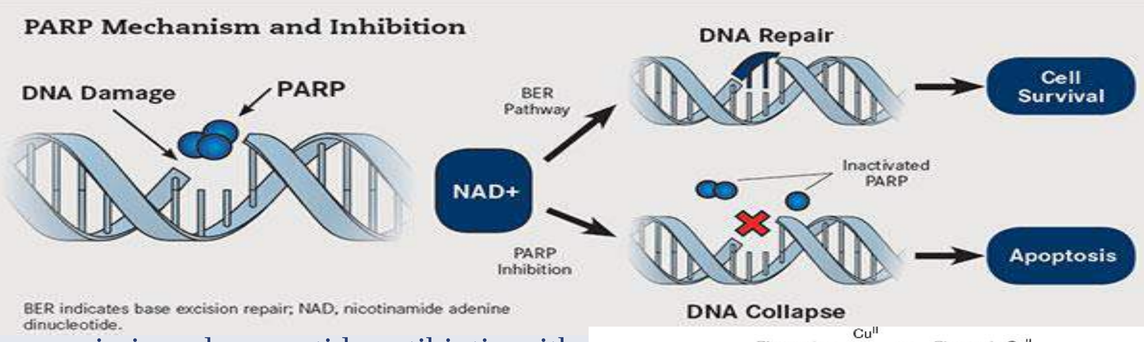
INTRODUCTION

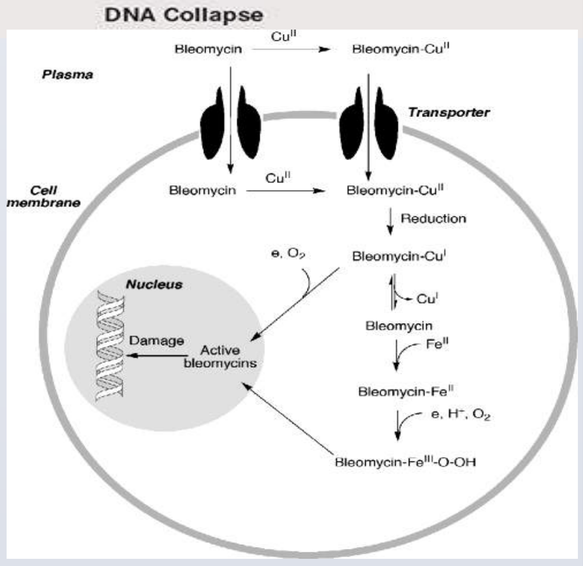
Cancer is the abnormal growth of cells which leads to proliferation in an uncontrolled way and, in some cases, to metastasize (spread). It is the second leading cause of death globally, and is responsible for an estimate of 9.6 million deaths in 2018. Globally, about 1 in 6 deaths happen due to cancer. It has effected India in a major way by causing 0.75 million deaths in 2018. Cervical cancer is the second most common cancer in Indian women accounting for 22.86% of all cancer cases in women.

According to the American Society of Cancer, high priority drugs used as cancer therapeutic are platinum-based. In India, lack of awareness and instrumentation leads to late diagnosis of cancer, compelling administration of higher concentration of platinum-based drug as therapeutics. The side-effect of using these drugs as a mode of treatment is development of Nephrotoxicity in large population of women, hence to overcome this situation, the use of another drug that would sensitize cancer cells towards platinum-based drugs would help reduce the effective concentration of it being used for treatment in patients.

Recent studies have shown that PARP inhibitors can be used as chemo-radiosensitizer in cancer patients, thus reducing the effective dose of Cisplatin (Platinum-based drug) being used. PARPi (Eg.: Rucaparib, Olaparib) actively bind PARP and stop cell's Base Excision Repair activity.



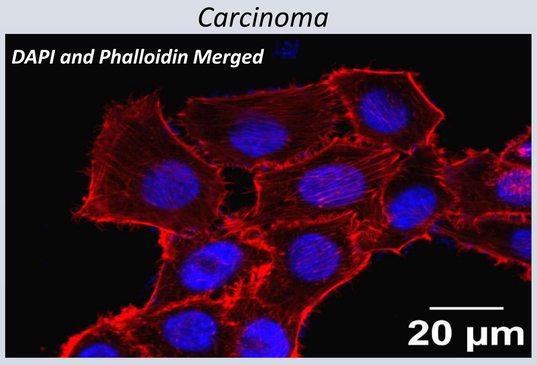
Bleomycin is a glycopeptide antibiotic with a unique mechanism of antitumor activity. It binds to GC-rich region of DNA after binding with divalent metals, such as, iron and copper. Molecular oxygen, bound by the iron, forms an active complex, that can produce highly reactive free radicals and Fe(III). The free radicals produce DNA single-strand and double strand breaks. As the outcome of using bleomycin is similar to that of radiations, we have used it as a radio mimetic agent for our studies.



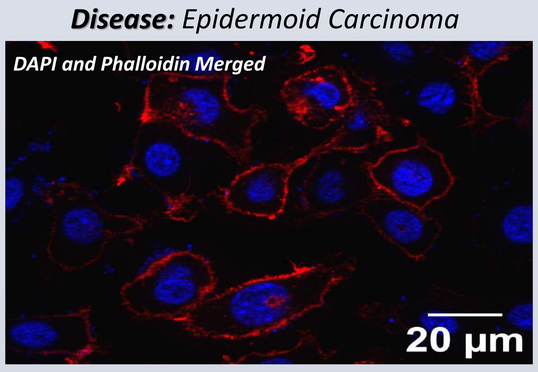
METHODOLOGY

❖ Human cervical cancer cell lines used for this study :

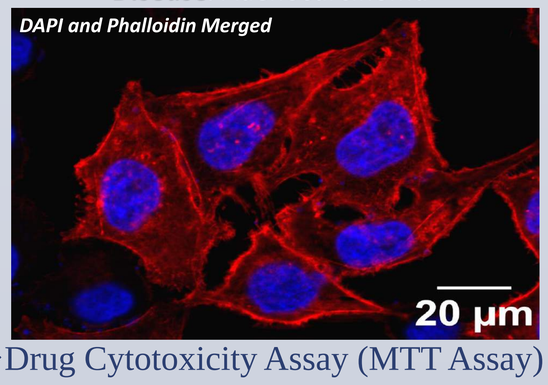
SiHa (ATCC® HTB-35™)
Organism: *Homo sapiens, human*
Tissue: *Cervix*
Disease: *Grade II, Squamous Cell Carcinoma*



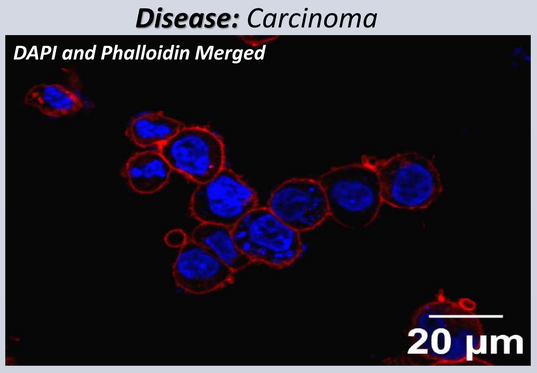
ME-180 (ATCC® HTB-33™)
Organism: *Homo sapiens, human*
Tissue: *Cervix;*
Derived From Metastatic Site: *Omentum*
Disease: *Epidermoid Carcinoma*



HeLa (ATCC® CCL-2™)
Organism: *Homo sapiens, human*
Tissue: *Cervix*
Cell Type: *Epithelial*
Disease: *Adenocarcinoma*



C-33A (ATCC® HTB-31™)
Organism: *Homo sapiens, human*
Tissue: *Cervix*
Cell Type: *Epithelial, Retinoblastoma*
Disease: *Carcinoma*

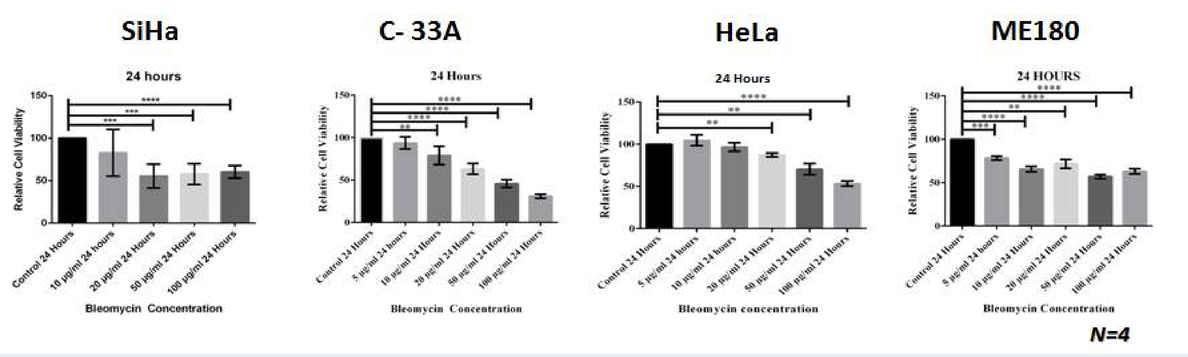


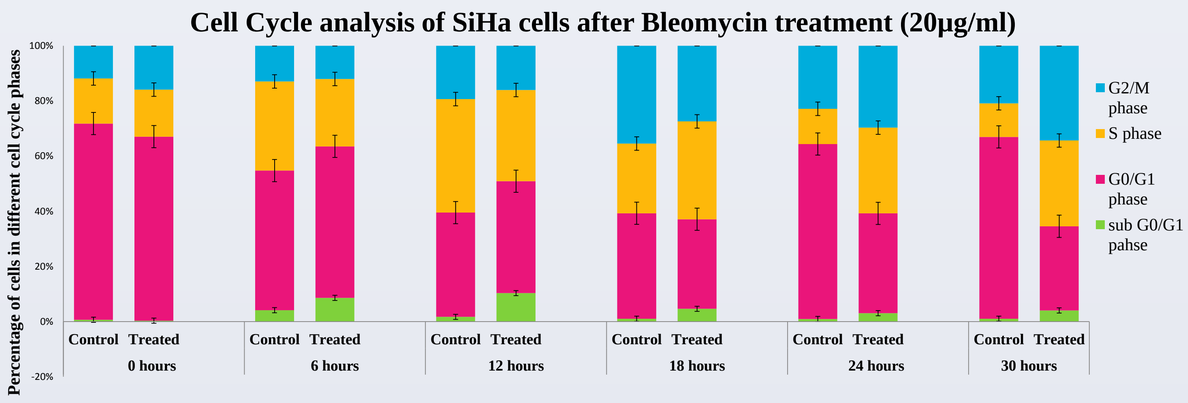
❖Drug Cytotoxicity Assay (MTT Assay)
❖Cell Cycle Analysis (FACS)
❖Bleomycin has been used as a radio-mimetic agent.

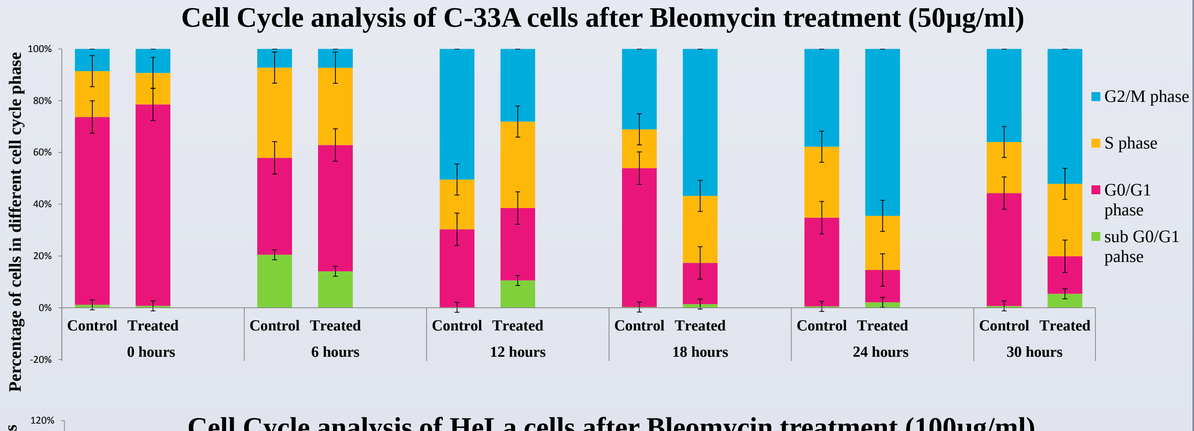
RESULT

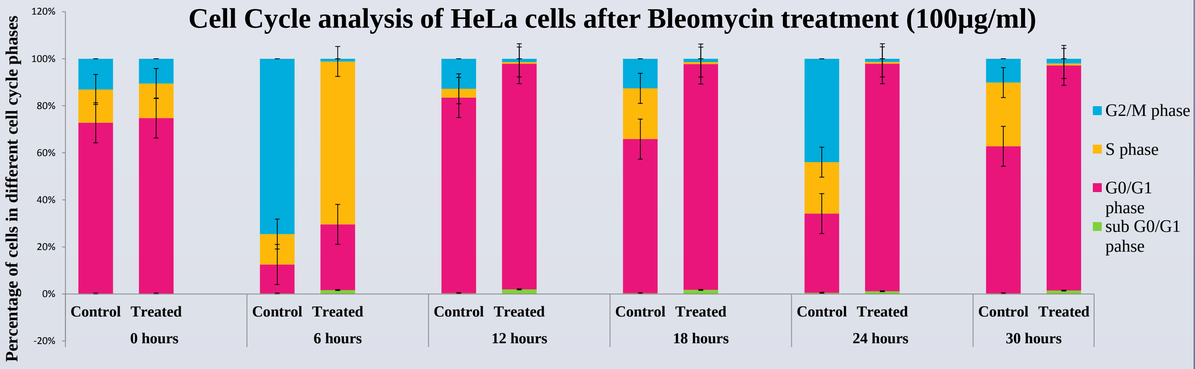
Differential sensitivity of cells to Bleomycin treatment

	SiHa	>	C-33A	>	HeLa	>	ME180
Observed IC50 values	20µg/ml		50µg/ml		100µg/ml		>100µg/ml









CONCLUSION & DISCUSSION

- ❑ Cell lines are differentially sensitive to Bleomycin.
- ❑ Administration of Bleomycin affects normal replication of these cells.
- ❑ The G2/M phase and S phase of cell cycle in Me-180 and SiHa gets elongated.
- ❑ HeLa cells get arrested at G0/G1 phase of cell cycle.

FUTURE PERSPECTIVE

❑Effects of Bleomycin on PARP expression needs to be analysed in combination with Cisplatin alone and then with Rucaparib.

❑Further characterization of cell lines needs to be performed.

❑After complete characterization and optimization of all the cell lines they will be injected into the SCID mice's thigh to generate cervical cancer.

❑Effect of Bleomycin, Cisplatin and Rucaparib on PARP expression and activity *in vivo* using cervical cancer mice model has to be studied.

ACKNOWLEDGMENT

The authors would like to thank Indian Institute of Science Education and Research, Kolkata for providing the facility to perform these experiments and Department of Biotechnology for funding the Systems Medicine Cluster (SyMeC) project.

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•Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. *J Natl Cancer Inst*. 2013;105(7):452-8.

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