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Confocal Microscopic Study of the Effects of Radiation Quality on the Interplay between the IGF1Rsignaling and the DNA Repair Pathway

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ABSTRACT

Ionizing radiation induces significant damage in the cell including chromosomal aberrations and mutations which if stable can induce cancer or leads to hereditary diseases. This biological damage depends on the radiation quality. Several studies have shown over expression of IGF1R in multiple cancer types including breast cancer. Activation of IGF1R initiates signalling pathways involved in cell proliferation, differentiation, survival and transformation. Some studies show that the apoptotic effect of IGF1R occurs by inhibiting the release of cytochrome c, through activating the PI3K/AKT pathway. Although some reports have shown that IGF1R delays DNA repair, it is not yet clear whether IGF1R delays DNA repair directly or through its ant apoptotic effects. Breast cancer cell lines MCF7 and MDA-MB-231 have been irradiated with gamma and alpha irradiation to study the relationship between radiation quality factor, DNA damage and kinetics of repair in breast cancer cells highly expressing IGF1R using immunofluorescence technique. In addition, investigation of the involvement of the RAD51 in DNA DSB repair in breast cancer cell lines has been done. The aim of this paper was to compare between breast cancer and normal cells response to the DNA DSB repair process, the DNA DSB repair pathway taken in breast cancer cells, and role played by IGF1R in the repair mechanism of DSB radiation induced damage.

Key words: Ionizing radiation, interplay, confocal microscopic.

Introduction

DNA double strand breaks (DSBs) can be caused both endogenously or exogenously. Endogenous oxidative stress induces primarily base damage and SSBs, but it can also induce DSBs. DSBs can arise after replication and during developmental process such as V (D) J recombination and meiosis. Two closely formed SSBs can form DSBs. DSBs can also arise at the critically shortened telomeres. Telomeres can be subjected to progressive shortening upon repeated division to an extent that perturbs its normal function; this may lead to the recognition of shorter telomeres as site of DSB which lead to the recruitment of DSB machinery.(d'Adda di Fagagna *et al.,* 2004; Jeggo and Lobrich, 2007; Khanna and Jackson, 2001; O'Driscoll and Jeggo, 2005; Verdun *et al.,* 2005) DSBs can be caused exogenously by DNA damaging agents such as ionizing radiation, chemical agent and UV light (Jeggo and Lobrich, 2007; Khanna and Jackson, 2001; O' Driscoll and Jeggo, 2005) . DSBs are biologically the most dangerous lesions produced by IR and other exogenous cytotoxic agents. Double-strand breaks are the essential menace to the genomic integrity of cells and, if incorrectly repaired or misrepaired, DSBs might lead to chromosome breaks, deletions, and translocations, it can cause apoptosis, permanent cell cycle arrest or mitotic cell death (Harmut, *et al.,* 2006 and Rothkamm *et al.,* 2003). If repaired incorrectly, DSB can lead to carcinogenesis through key gene inactivation, chromosomal translocations, inversion or deletion, or the generation of unstable chromosomal abnormalities (Bradbury, 2003; Fernandez *et al.,* 2004 and Jeggo and Lobrich, 2007).

There are two distinct DNA DSB repair pathways Homologous recombination (HR) and Non-Homologous end joining repair pathway (NHEJ). HR is an error free repair pathway that uses the sister chromatid as a template and dominates mostly in M-phase, whereas, the error prone, NHEJ is likely to occur during M1 and S-phase (Bassing *et al.,* 2002; Celeste *et al.,* 2002; Ismail and Hendzel, 2008).

Many studies have suggested a direct role of IGF1R in DNA repair enhancement, one of which proposed a role of IGF1R phosphorylation in RAD51 recruitment. RAD51, a repair protein involved in homologous recombination repair pathway, is recruited to the site of damage after ligand binding phosphorylation of IGF1R, which sends signals to insulin receptor substrate one (IRS-1), which in turn mediates the pre-nuclear recruitment of RAD51 to the site of damage (Trojanek, *et al.,* 2003). In another study, Phosphorylated IGF1R has been proven to play a role in DNA damage repair via a complex interplay between signaling intermediates, PI3 kinase and p21 (Clark, *et al.,* 2005).

Many previous studies have indicated that inhibition of the phosphorylation of IGF1R induce cell death, decrease cell viability, and increase radio sensitivity. In this paper immunofluorescence technique was used to

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study the mechanism by which breast cancer cells repair the radiation induced DSB, experiments have been conducted in over expressing and impaired IGF1R breast cancer cells.

Materials and Methods

Cell lines:

Frozen MCF10A cell line were commercially purchased from ATCC and following retrieval from liquid nitrogen, the cells were re-seeded in 25cm^2 flask in Dulbecco's modified Eagle's medium (DMEM)/ ham's F-12 supplemented with 5 % horse serum, 10 µg/ml human insulin, 10 ng/ml EGF, 100 ng/ml cholera toxin, 0.5µg/ml hydrocortisone, 100 units/ml of penicillin/ streptomycin sulphate, and 0.01% L-Glutamine. The cells were left to grow to 70% confluent in humidified atmosphere of 95% air, 5% CO_2 , tripsinized with 0. 5% trypsin and then transferred into 75 cm^2 falcon flasks.

Frozen MCF7 and MDA- MB-231 were obtained from Laura Philips a DPhil student in Ruth Michel's group at the Gray Institute, The University of Oxford. Cells retrieved from liquid nitrogen, were seeded in 25cm^2 flasks into DMEM medium supplemented with 10% fetal bovine serum, 10 units penicillin/streptomycin and 0.01% L-Glutamine. Cells were left to grow to 70% confluent in humidified atmosphere of 95% air, 5% CO_2 , tripsinized with 0.25% trypsine and then transferred into T75 flask.

Irradiation facilities:

Cells were irradiated with either ^{137}Cs γ–rays which is considered as low linear energy transfer (LET) ionizing radiation or Pu -238 α-particles, as an example of high LET. Monolayer cells were irradiated at room temperature with ^{137}Cs γ–rays at a dose rate 1.94 Gy/min, and then transferred after irradiation onto an iced surface where the medium was changed immediately. The cells were kept at 37^{o}C in incubator for a given time depending on the endpoint to be determined. Cells were irradiated with α-particles usingPu-238, (LET is approximately 129 KeV/µm if a cell thickness of 5 µm is assumed). Pu-238 with energy 3.2 MeV is produced in reactors by bombarding uranium oxide with deuterons. Prior to irradiation, cells were seeded either onto a 0.9 µm mylar-based dish. To help MCF10A cells to attach as a monolayer population, the mylar surface was initially treated with Cell Tak cell adhesive. Before seeding, Cell Tak was added to dishes at a density of 3µg cm^{-2} of surface area in 0.1 M sodium bicarbonate buffer for 45 min then washed three times with 1X PBS. Cells were irradiated at room temperature then dishes are transferred to an iced cold surface before changing the medium. The cells were incubated at 37^{o}C.

The mean nuclear area of MDA-MB-231 and MCF10A cells were measured before and after irradiation using confocal microscope (Zeiss LSM 710). Cells were stained with rhodamine-123 to identify the nucleus of the attached cells. Nuclear area measurements were used to calculate the average number of α- particles track traversal per nucleus using the following equation:

N= (DA/0.16L),

Where N is number of α- particles traversals, L is linear energy transfer (LET, keV/µm), A is nuclear area (µm^2) and D is dose in Gy (Portess *et al.,* 2007).

Flow cytometric analysis of AG538 treated breast cell lines:

Cells were fixed in 0.5 ml iced-cold 70% ethanol was added drop wise with vortex and the cells left at 4^{o}C for at least 30 min.

Cells were centrifuged at 250g for 5 min at room temperature and then incubated with 20 µl propidium iodide and 20-100 µg/ml in 1xPBS at 37^{O}C for 20-30min. The DNA content of the cells was then analyzed using a BD Becton Dickinson FAC sort analyzer. DNA content throughout the cell cycle was quantified using ModFit software.

Immunofluorescence staining:

Cell fixation and staining:

Cells were washed with 0.5 ml 1x PBS and then fixed in 0.5ml 3% paraformaldehyde (in 1xPBS) for at least 30 min. Cells were then washed three times with 0.5 ml 1xPBS each wash for 5 min Then premeabilised in 200 ul 1% triton (1xPBS) for ten minutes. Cells were blocked with 200 ul 1% BSA and 1% FSG (in 1xPBS) for at least one hour. Cells were stained with the primary and then with secondary antibodies, and a drop of hard set vectashield plus Dapi added and finally a cover slip is placed on the top. Cells were kept in the dark in the

fridge until detection under Zeiss-710 confocal microscope. The images were analyzed using Zen 2008 LSM software for foci detected fluorometrically.

Treatment of cells with the IGF1R inhibitor AG538:

AG538, an IGF1R inhibitor, was dissolved in 1ml DMSO to make a stock solution of 16.8 mM and 1ml aliquots were stored at -20°C.

For both gamma and alpha irradiation, seeded cells were treated with 75uM AG538 for one hour and control cells were treated with DMSO alone. Cells were then irradiated with 0 and 6 Gy, incubated at 37°C then collected at the same time point as for γH2AX and RAD51 kinetics experiment. The cells were then fixed and stained as described above.

Results And Discussion

Dose dependence of the formation of DNA damage in irradiated cells:

Gamma rays irradiated cells:

Figure (1) represents the dose dependent yield of γH2AX (DSB) and RAD51 foci measured one hour following γ-irradiation, where γH2AX is used as a biomarker for DNA DSB (Djuzenova *et al.,* 2013 and Mah *et al.,* 2011) and RAD51 is used as a biomarker of the homologous recombination repair pathway (Banáth *et al.,*2010)

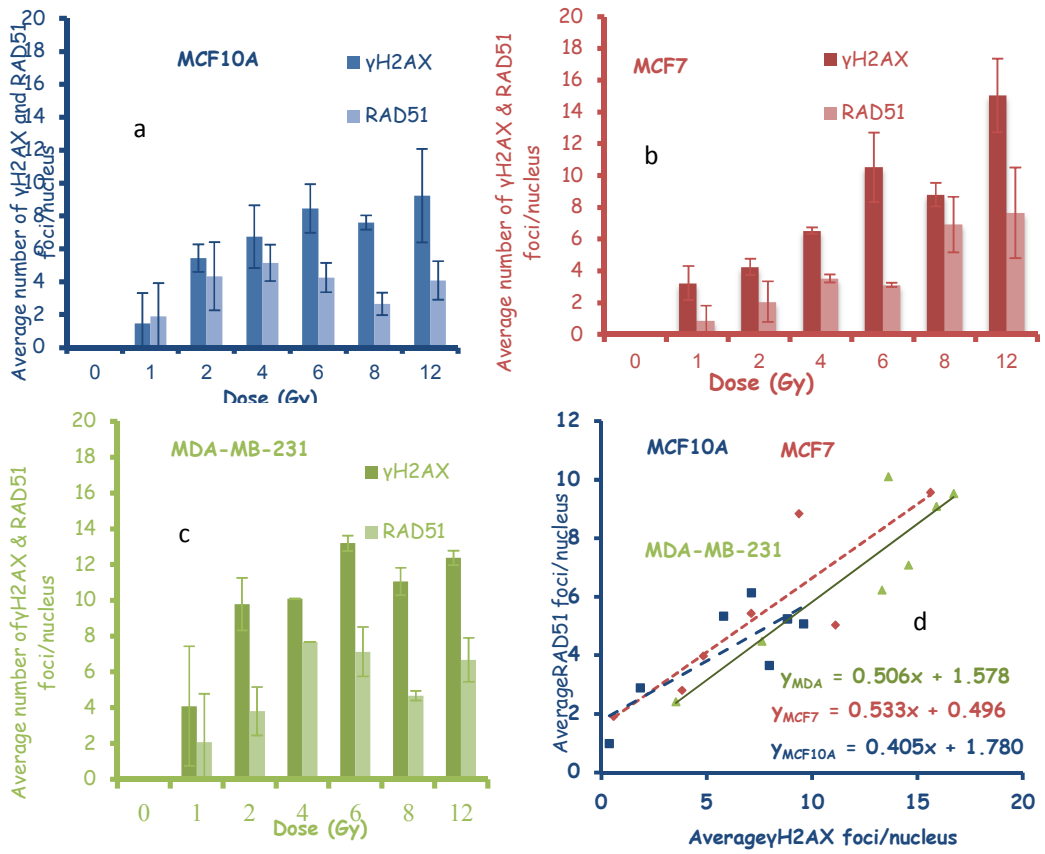

Fig. 1: The dose response for the yield of γH2AX (dark) and RAD51 (light) foci 60 min after γ-irradiation of the breast control cell line MCF10A(a), MCF7 (b), and MDA-MB-231(c)and linear correlation between γH2AX and RAD51 yield after γ-irradiation for the three cell lines (c) The data represent the mean of two replicates experiments.

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The 60 min has been chosen because it is assumed that the more persistent DSB will remain and most of breaks will have been re-joined. Results of dose response for the three cell lines show an increase in number of γH2AX foci (DSB) and RAD51 with dose that reach a plateau after approximately 6 Gy for MCF10A and MDA-MB-231 but no detectable plateau for MCF7, furthermore, after we draw the relation between γH2AX and RAD51 in figure (1d) for the three cell lines, data observed show a linear fit relationship with nearly the same slop for MCF7 and MDA-MB-231 (0.5) and a smaller slop for MCF10A (0.4).

Alpha particles irradiated cells:

Figure (2) represents the dose dependent yields of γ H2AX or RAD51 foci tracks determined one hour following α-particle irradiation. γ H2AX is used as a biomarker for the DNA DSB and RAD51 is used as a biomarker of the homologous recombination repair pathway. The yields of foci tracks for γ H2AX at 1 Gy is similar to the number of radiation tracks determined for 1 Gy based on the morphology of the cells.

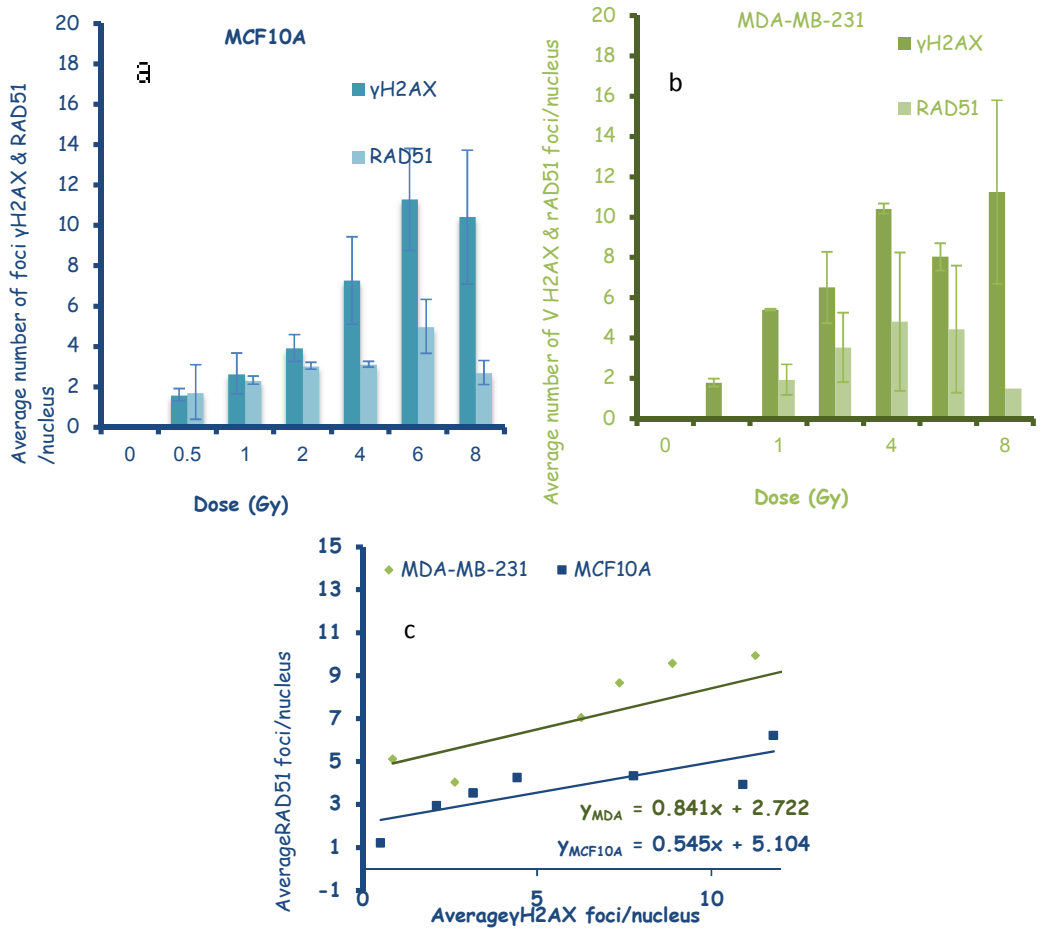

Fig. 2: The dose response for the yield of γH2AX (dark) and RAD51 (light) foci 60 min after α-particle irradiation of the breast control cell line MCF10A(a)and MDA-MB-231(b)and linear correlation between γH2AX and RAD51 yield after γ-irradiation for the three cell lines(c). The data represent the mean of two replicates experiments.

Kinetics of DNA DSB repairs after irradiation

Gamma irradiated cells:

Figures (3) represent the average no of γH2AX and RAD51 foci per nucleus detected at different times (0-280 min) after exposure of breast cancer cells MCF7 and MDA-MB-231and the control MCF10A cells to 2 Gy γ-radiation. After irradiation, cells were co-stained with anti-γH2AX and anti-RAD51 primary antibodies.

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Alpha particles irradiated cells:

γH2AX assay has been performed for breast cancer cells after their exposure to 2 Gy of α- particles to study the kinetics of DSB loss up to 48 hours of irradiation. Moreover, RAD51 assay was used to study the HR repair pathway. Due to the irradiation configuration, the fluorescently detected DNA damage in cells is seen as foci tracks with α-particle irradiation. Figures (4) represent the average number of γH2AX and RAD51 foci per nucleus at various times after irradiation of breast cancer cells MDA-MB-231and its control MCF10A with 2 Gy of alpha particles.

The DNA content of breast cancer cells after treatment with IGF1R inhibitor:

the cells were serum starving for 24 hours. The MCF7 and MDA-MB-231 cell lines were treated with 75μM AG538 or DMSO as control for one hour, prior to irradiation with 6 Gy gamma rays at room temperature and subsequent collection at 15, 30, 60, 120, 240, 360 and 1440 minutes after irradiation. Cells were fixed in iced cold 70 % Ethanol and then stained with propidium iodide at 37^{O}C for analysis by FACsort analyser. Figures (5 a and b) show the cell cycle distribution for selected time points with and MDA-MB-231. Figures show that cells were seen to arrest in G1 phase.

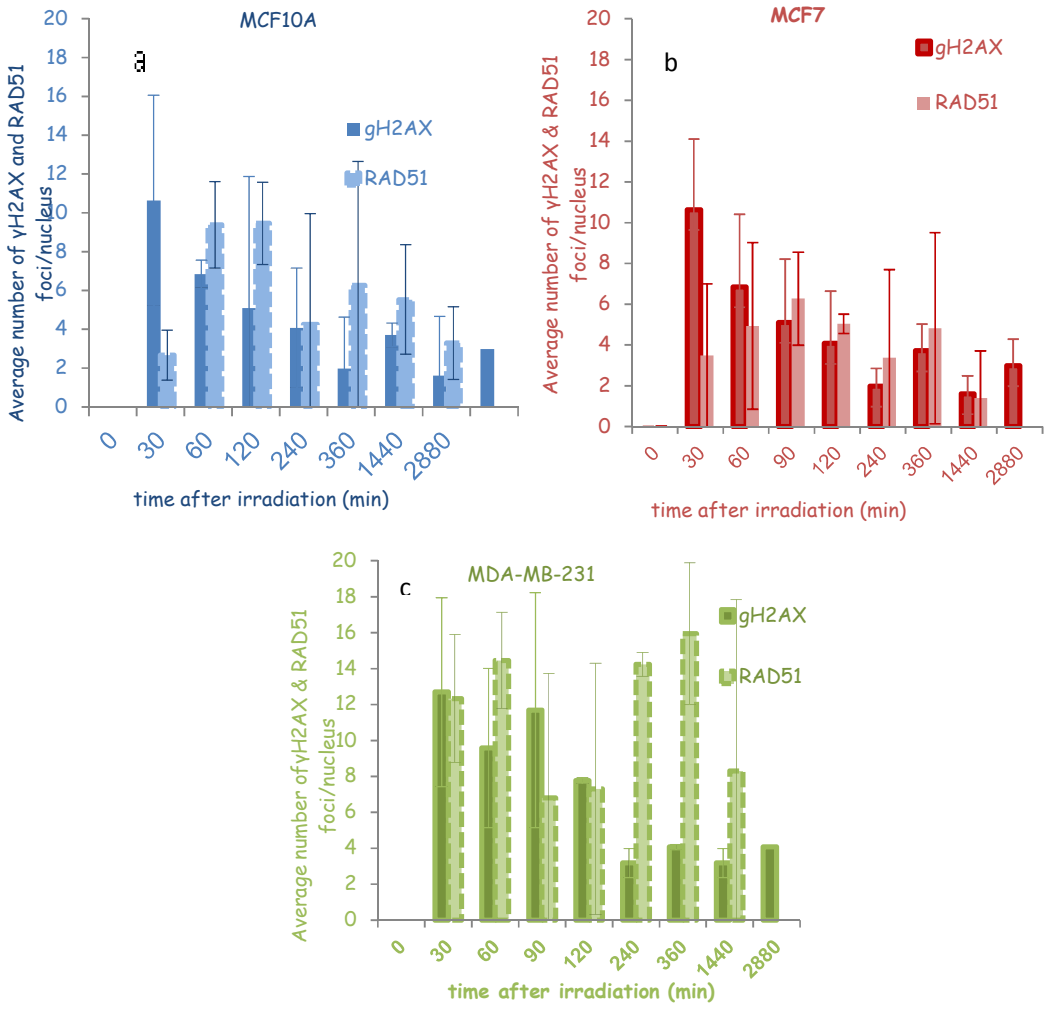

Fig. 3: Shows the repair kinetics of radiation-induced damage detected as γ H2AX (dark) or RAD51 (light) foci at various times after γ -irradiation of the control cell line MCF10A (a), MCF7 (b) and MDA-MB-231 (c) with a dose of 2 Gy for three replicate experiments.

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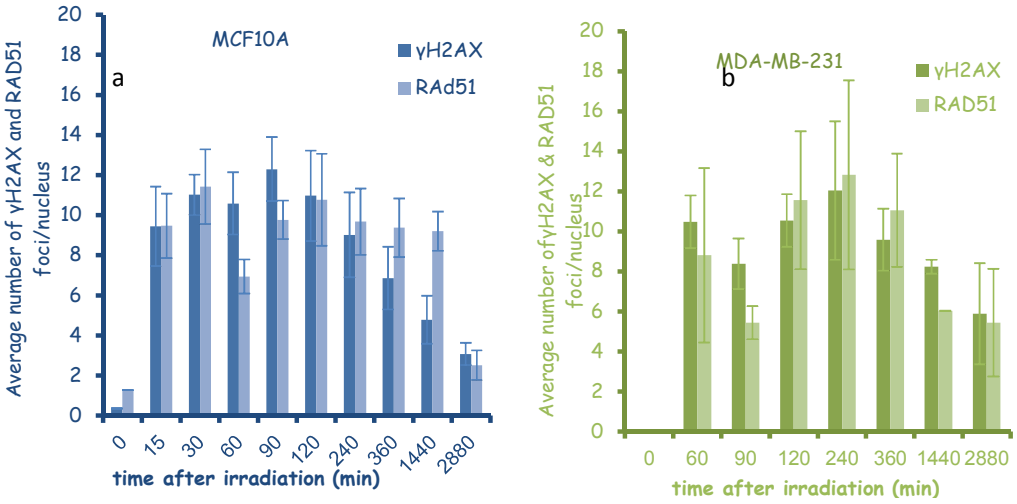

Fig. 4: The repair kinetics of radiation-induced damage detected as γH2AX (dark) or RAD51 (light) foci at various times after α- particles irradiation of the control cell line MCF10A with a dose of 2 Gy for three replicate experiments (a) and for the breast cancer cell line MDA-MB-231 (b).

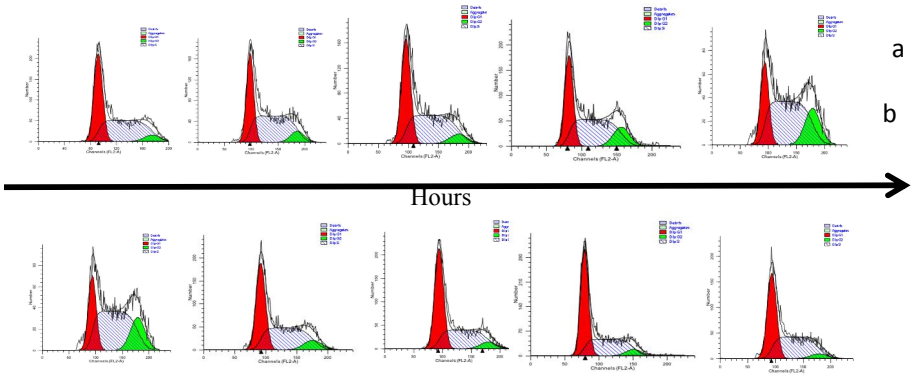

Fig. 5: DNA content analysis for MDA-231 after DMSO (a) and IGF1R Inhibitor (b) treatment and irradiated with 0 and 6 Gy of γ-radiation. Time points were 1, 2, 6 and 24 hour after irradiation.

Kinetics of DNA DSB repairs after the inhibition of IGF1R:

Gamma rays irradiated cells:

Cells have been treated with 75μM AG538 or DMSO, the inhibitor solvent, for one hour at 37^{O}C, irradiated with 6 Gy gamma irradiation then collected at 15, 30, 60, 90, 120, 240, 360, 1440, 2880 min after post-irradiation incubation at 37^{o}C. The collected cells were co-stained with anti-γH2AX and RAD51 Immunofluorescence primary antibodies. Figures (6) show the kinetics of DNA DSB repair and the recruitment of RAD51 after gamma irradiation of breast cancer and control cell lines treated with either AG538 or DMSO.

Alpha particles irradiated cells:

After conducting experiments on the effect of AG538 on the kinetics of DSB repair in breast cancer cells and their control using low LET α-radiation, we aimed here to compare our results with the alpha radiation which is high LET radiation.

We used the same dose of radiation, 6 Gy, as used in the γ-radiation studies and as shown to stimulate IGF1R. This stimulation is dependent on radiation dose with the phosphorylation of IGF1R reaching a peak at 6 Gy.

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Figures (7) show the kinetics of DSB repair in breast cancer cells MDA-MB-231 and its control MCF10A after treated with 75µM AG538 or DMSO at 37^{O}C for an hour then exposed to 6 Gy alpha radiation at room temperature and then incubated at 37^{O}C for various times upto 48 h.

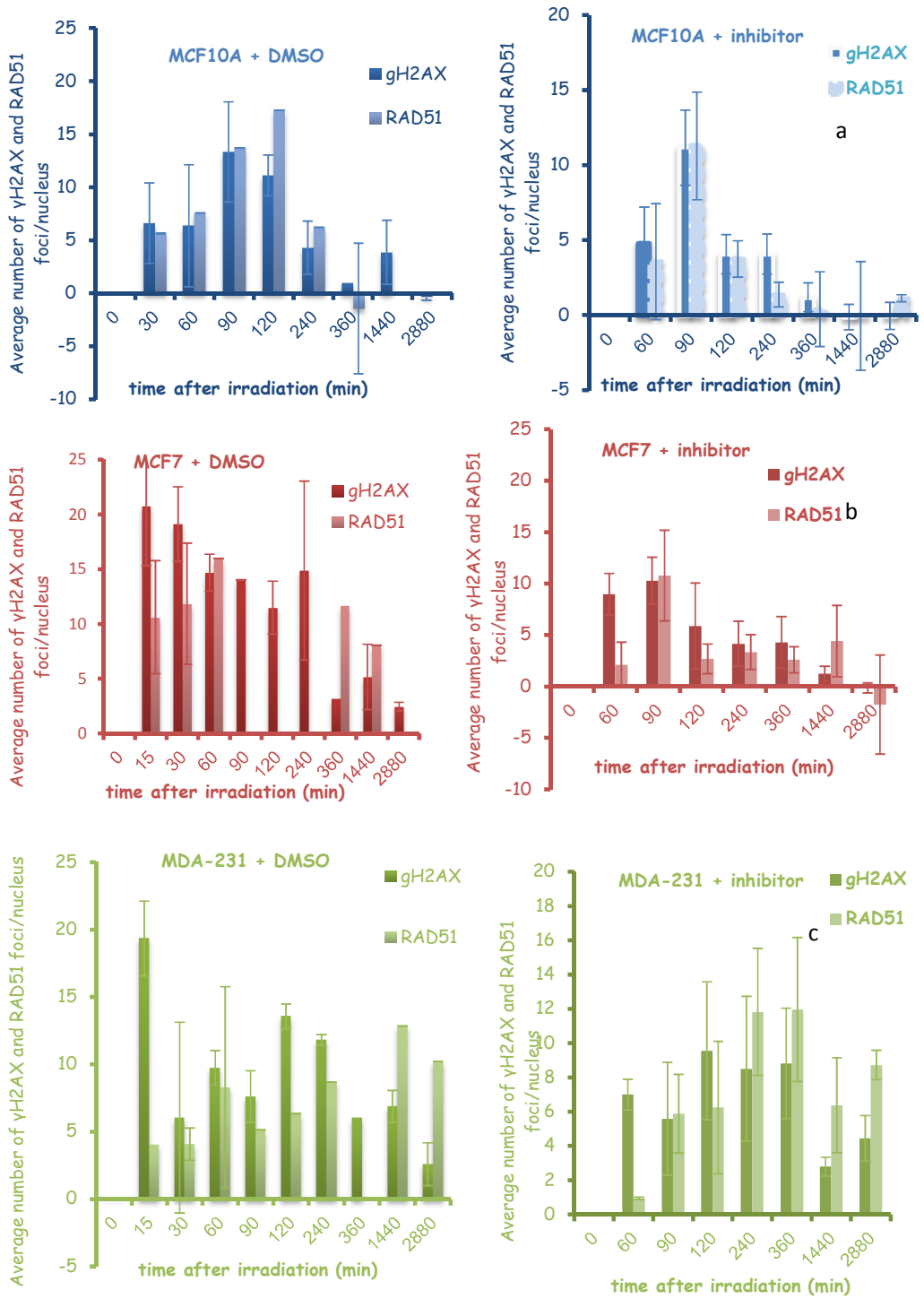

Fig. 6: The repair kinetics of DNA DSB for the control breast cell line MCF10A (a), breast cancer cell line MCF7 (b) and MdA-MB-231 (c) after exposure to 6 Gy gamma radiation for three replicate experiments, Cells were treated with DMSO (left Panel) and AG538 (right panel) for an hour prior to irradiation.

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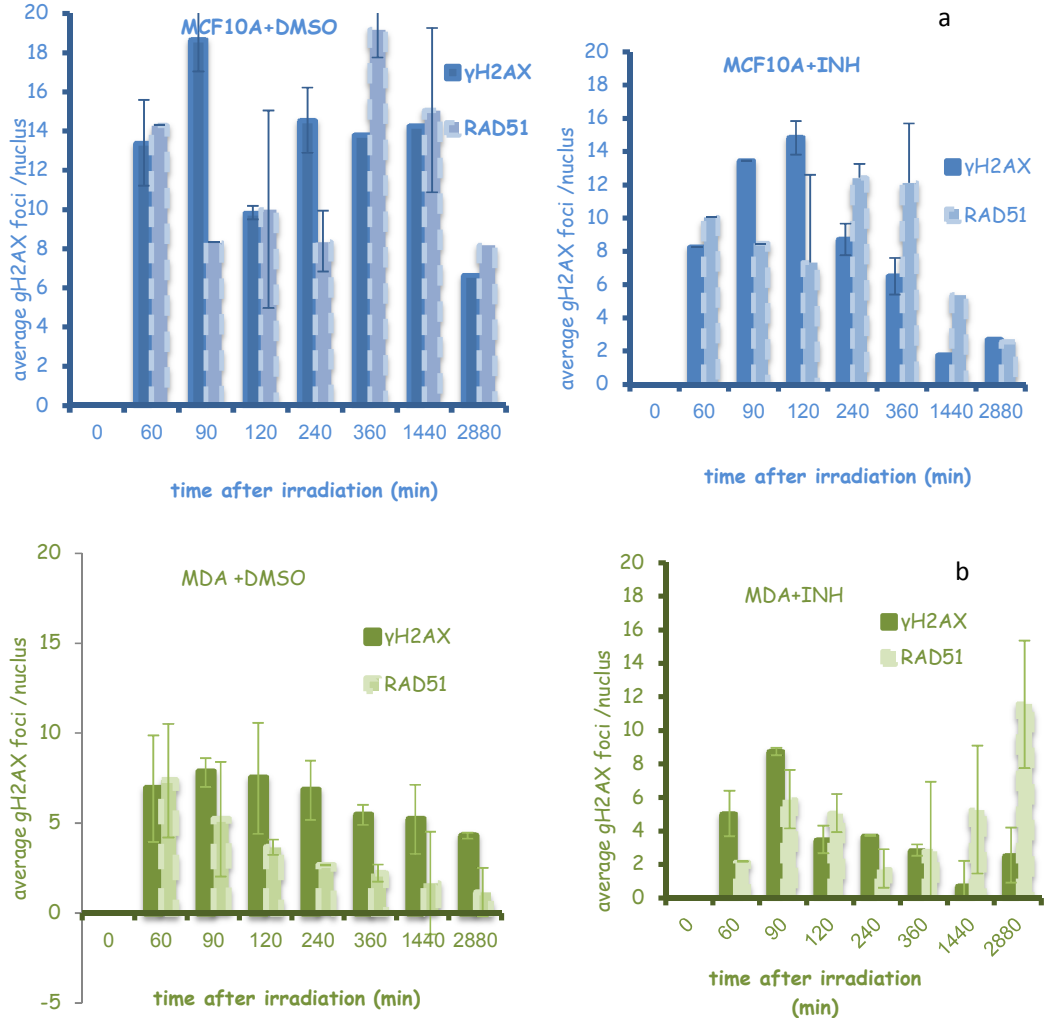

Fig. 7: The repair kinetics of DNA DSB for the control breast cell line MCF10A (a) and MDA-MB-231 (b) after exposure to 6 Gy α-particles radiation for three replicate experiments, Cells were treated with DMSO (left Panel) and AG538 (right panel) for an hour prior to irradiation.

The dose response show dependent yield of γH2AX (DSB) and RAD51 foci measured one hour following γ-irradiation, where γH2AX is used as a biomarker for DNA DSB and RAD51 is used as a biomarker of the homologous recombination repair pathway. The 60 min has been chosen because it is assumed that the more persistent DSB will remain and most of breaks will have been re-joined. Results of dose response for the three cell lines show an increase in number of γH2AX foci (DSB) and RAD51 with dose that reach a plateau after approximately 6 Gy for MCF10A and MDA-MB-231 but no detectable plateau for MCF7, furthermore, after we draw the relation between γH2AX and RAD51 in figure (1a) for the three cell lines, data observed showed a linear fit relationship with nearly the same slop for MCF7 and MDA-MB-231 (0.5) and a smaller slop for MCF10A (0.4). the alpha particle dose response show that the yields of foci tracks for γH2AX at 1 Gy is similar to the number of radiation tracks determined for 1 Gy based on the morphology of the cells. Data show an increase in γH2AX yield and RAD51 foci with dose that reach a plateau after 4 Gy, moreover, the relationship between γH2AX and RAD51 fit to a linear coloration, figure (2c), with a higher slop value for MDA-MB-231 compared to MCF10A, furthermore, the slop value of α-particles irradiated cell lines show higher values compared to the corresponding gamma irradiated ones.

The DSB kinetics in MCF10 cells show the appearance of fifteen DSB, detected as γH2AX foci within 30 min after irradiation of MCF10A cells. The yield of DSB decreases with time with <5 DSB persisting twenty four hours after irradiation. The level of RAD51 foci at early times is less than that for γH2AX but is similar to that γH2AX at 24 h. the damage induced in MCF7 cells is less than that in MCF10A cells at early times., The yield of RAD51 foci after irradiation sis similar to the yield of DSB detected as γH2AX at times up to twenty

four hours. The yield of DSB at different times after irradiation of MDA-MB-231 cells with 2 Gy of radiation. The initial yield of γH2AX foci at early times then decreases representing repair of DSB with time. Moreover, the figure show the slight increase in RAD51 foci until it peak after six hours after irradiation and stay high until twenty four.

We have shown that exponentially growing MDA-231 breast cancer cells form more DSB damage compared with the yield induced in MCF7 cells after exposure to low LET γ-radiation. The number of γH2AX foci in MDA-231 cells corresponds to eighteen foci after 30 min following 2 Gy γ-irradiation compared with the maximum number of five foci in MCF7 cells seen at 15 min post-irradiation. This result is not consistent with that obtained by Cai, *et al.*(2008)after γ-irradiation of MDA-231 and MCF7 cell lines. Their work results showed that after 30 min of 1Gy γ-irradiation, higher levels of γH2AX foci were observed in MCF7 compared with MDA-231 Moreover, in other studies by Georgakilas et al, 2008, results showed that the level of γ-H2AX after 30 min in MCF7 exposed to 5Gy of γ-irradiation reaches ~ 25 foci/Gy, equivalent to 5 γ-H2AX foci/Gy. The differences between our results and their results considering the number of foci formed in MCF7 cell line after irradiation may in part reflected by counting foci per nucleus, whereas, the results of Cai, *et al.*(2008) and Georgakilas *et al.* (2008) are based on estimating the intensity of γ H2AX/nucleus without subtraction of background intensity.

The repair of DSB induced by γ-radiation in exponentially growing MDA-231 cells is slower than that seen with MCF7 cells. Some DSB (around 5 γ H2AX/cell) are still present even after forty eight hours of irradiation. From comparison of loss of γ H2AX foci with time in all three cell lines, the repair kinetics in MDA-231 cell line show a shoulder after ~2 h but this disappears by six hours post-irradiation.

our results are consistent with the results of Cai, *et al.*(2008), who found that a number of the MDA-231 DSB remain non-repairable after 20 h following 10 Gy gamma irradiation compared with MCF7 cell line where the majority of DSB are repaired by 20 h (Cai, *et al.,* 2008). From these results it is inferred that the repair mechanism of some of the DSB induced by low LET radiation in MDA-231 cells is not as efficient as in exponentially growing MCF7 cells. This raises the question whether the reparability of some of the more complex types of DSB is compromised.

With alpha irradiation γH2AX is likewise recruited to sites of DSB within 30 min after irradiation but shows a gradual increase until the yield of γH2AX peaks four hours post-irradiation. A residual yield of γH2AX is indicative of some DSB remaining un-repaired forty eight hours post-irradiation. A small fraction of DSB is repaired slowly in both exponentially growing control MCF10A and breast cancer MDA-231 cell lines. This data indicate that high LET irradiation cause more persistent DSB in both cell lines compared with low LET gamma irradiation. Although only a slight difference is seen between the levels of non-repairable DSB residues twenty four hours post alpha irradiation in both cancer cell line MDA-231 and the control cell line MCF10A, the MCF10A cells are more radiosensitive compared with MDA-231 after alpha irradiation.

Considering γH2AX for both low LET gamma rays and high LET alpha particles, results indicate that the exponentially growing MDA-231 have more residual DSB. Further this cell line shows slower rates of DSB repair. This observation may indicate that complex DSB formed are not so readily repaired or that the repair machinery is not so effective at repairing more complex DSB.

Results after gamma and alpha particles irradiation showed that RAD51 is involved in the repair process in exponentially growing MDA-231 and MCF7 cells through the observation of RAD51 foci in the cells. This observation implies that the homologous recombination pathway is involved in part in the repair of DSB induced in breast cancer cells and that RAD51 might play a role in exponentially growing cell line that over express IGF1R.

Previous work by Krzysztof Reiss et al, 2003, has shown that HRR is enhanced in cells expressing IGF1R depending on the level of the receptor, in a response that is controlled at the level of RAD51 translocation to the nucleus (Trojanek, *et al.*, 2003). It was suggested that a direct interaction of RAD51 with insulin receptor substrate 1, IRS-1 is involved. MCF7 cell lines have reduced expression of BRCA1 and in a study by Eva Y-H.P.lee *et al.*(1999) they showed that mutant BRCA1 cell lines are proficient in RAD51 formation (Kachhap, Vetale *et al.* 2001)(Yuan, *et al.* 1999).

The kinetics of DNA DSB repair and the recruitment of RAD51 after gamma irradiation of breast cancer and control cell lines treated with either AG538 or DMSO. It can be seen that the rate of repair of damage, based on loss of γH2AX foci, in MCF10A and MCF7 cells is faster comparing with that in MDA-MB-231 cells. From the time course of loss of RAD51 foci, the figures show that in cell line MCF10A and MCF7 the loss is similar to the loss of γH2AX foci. With MDA-MB-231 cells it can be seen that the recruitment of RAD51 is delayed and that the number of RAD51 foci is lesser than that for γ H2AX foci.

Comparing our data from control cells treated with DMSO or with the inhibitor AG538, figures (5-36, 5-38, and 5-40) show that the number of γH2AX foci is reduced for all cell lines and the rate of repair of DSB is faster. The same was seen for RAD51 foci except with MDA-MB-231 cells. Figure (5-41) shows that RAD51 is recruited to the site of damage with the number of foci remaining high up to later time, showing the involvement of its role in the process of repair for long time.

In the presence of the inhibitor of IGF1R results show that the number of DSB is reduced and the rate of repair of DSB is faster in both cell line. Additionally, a reduction in the level of recruitment of RAD51 occurs but the number of RAD51 foci increases up to 48 hours after irradiation in the MDA-MB-231 cell line.

Results show that inhibition of IGF1R using 75μM AG538 on quiescent MCF7, MDA-231 breast cancer and MCF10A control cell lines causes a reduction in DSB formation as well as an enhancement of the rate of repair of DSB induced by gamma and alpha particle irradiation for both normal and malignant breast cell lines. The reduction in the number of DSB in quiescent cells could reflect slower rate of replication as most cells are arrested in G1 phase, (Trojanek, *et al.*, 2003).

However, several reports have indicated a relationship between the activation of IGF1R and the efficiency of DNA DSB repair. It was suggested that a direct role of phosphorylated IGF1R in enhancing DSB repair through HRR or NHEJ pathway or an indirect role of IGF1R through an anti-apoptotic effect. From results it is implied that inhibition of IGF1R phosphorylation cause the rate of repair of DSB to be enhanced and the yields of residual DSB or replication-induced DSB, those present 24-48 h post-irradiation, to decrease consistent with an increased efficacy of damage repair.

Additionally, results show that the recruitment of RAD51 co-localized DNA DSB is similar whilst the kinetics of loss of RAD51 foci after gamma and alpha particle irradiation is similar to that for loss of γH2AX foci. It is inferred that RAD51 is involved in the repair of some DSB in control and malignant breast cell lines in the presence of the inhibitor of IGF1R with a larger role for RAD51 with MDA-231. Previous reports suggested that the phosphorylation of IGF1R enhances DNA DSB repair through IRS-1 mediated activation of Ras-mitogen-activated protein kinase (MAPK) signal pathway. In this pathway, it was suggested that activated IGF1R signals to IRS-1, and as a consequence facilitates nuclear recruitment of RAD51 (Trojanek, *et al.* 2003). The studies of Dricu *et al.*(2007) showed that inhibition of IGF1R blocks radiation induced Ku activation leading to a reduction of nuclear DNA-Ku binding level and nuclear Ku-86 down regulation. As a consequence these effects cause radiosensitization of lung cancer cells which are known to over express IGF1R. The study with breast cells showed recruitment of RAD51 to the site of damage even when IGF1R phosphorylation is inhibited (Cosaceanu, *et al.* 2007).

In conclusion, we have shown that high LET alpha particles are less biologically effective on breast cancer cell line MDA-231. Moreover, the process of DSB repair is slow and a number of DSB remain unrepaired even after 48 hrs of irradiation especially for high LET radiation. Inhibition of IGF1R in serum starved breast cancer cell lines MCF7 and MDA-231 although decreasing the viability of cells, enhance the process of DSB repair.

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