3 - 75 First Interdisciplinary Experiment Using High Energy Microbeam

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The high energy ion beam of tens to hundred MeV/u possesses mm-to-cm penetration depth in materials and can be easily extracted into air without significant energy loss and beam scattering. Combination of high energy ions and microbeam technology facilitates the microprobe application to many practical studies in large scale samples. The IMP heavy ion microbeam facility has recently been integrated with microscopic positioning and targeting irradiation system. The first interdisciplinary experiments performed at the IMP microbeam facility using the beam of 80.5 MeV/u carbon ions include bystander effect study, irradiation effect on nerve system, security algorithm attack study. Bystander effect induction via medium transferring was not found in the micro-irradiation study using HeLa cells. The mouse irradiation experiment demonstrated that carbon irradiation of 10 Gy dose to its tuberomammillary nucleus did not impair the sleep nerve system. The fault injection attack on RSA (Rivest–Shamir–Adleman) decryption proved that the commercial field-programmable gate array chip is vulnerable in single event effect to low linear-energy-transfer carbon irradiation, and the attack can cause the leakage of RSA private key(Fig. 1). These experiments demonstrate the potential of high energy microbeam in its application to biology, biomedical, radiation hardness, and information security studies.

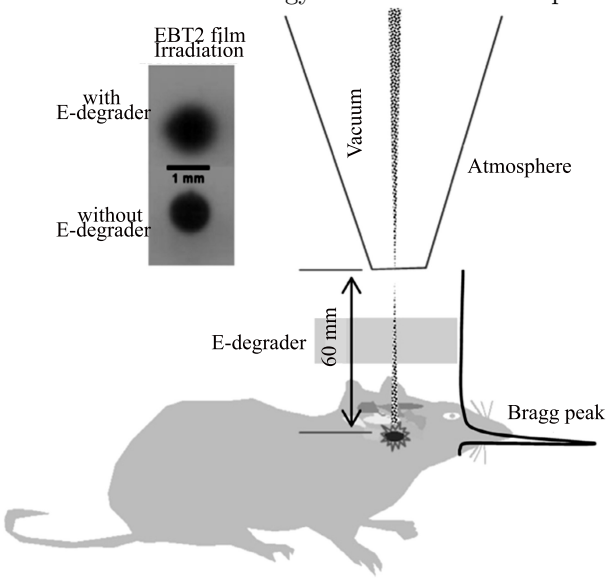

Fig. 1 Raster scan irradiation of mouse nerve nucleus using high energy microbeam of 80.5 MeV/u carbon ions. The upper-left insert shows the photo scan of EBT2 film raster-scan irradiated with and without energy degrader.

3 - 76 Kinetics of DNA Repair Proteins 53BP1 and γ-H2AX in HeLa Cells Irradiated with X-rays

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Double-stranded DNA breaks (DSBs) are the most lethal type of DNA damage, therefore, the inefficient or inaccurate repair can create mutations and chromosomal translocations which induce genomic instability[1]. DSBs can be induced by many events such as ionizing radiation, reactive oxygen species and radiomimetic drugs. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the main DSB repair pathways[2]. It has been reported that histone variant H2AX phosphorylation (γH2AX formation) at DSB sites is required for the accumulation of many DNA damage response and repair proteins such as 53BP1[3]. 53BP1 is an established player in the cellular response to DNA damage[4]. This work investigated the kinetics of ionizing radiation induced foci (IRIF) of both proteins in HeLa cells irradiated with 0.2 and 1.0 Gy X-rays.

HeLa cells were kindly provided by Dr. Zhou (Institute of Modern Physics, CAS, China) routinely maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin and 100 U/mL penicillin. Samples were fixed in 4% paraformaldehyde for 10 min and in methanol at -20 ° C for 20 min, blocked with 5% blocking buffer for 2 h and stained with primary antibodies (mouse anti-γ-H2AX, #ab26350 and rabbit anti-53BP1, #ab36823, Abcam) for 2 h. The primary antibody was visualized using goat anti-rabbit labeled with Rhodamine or goat anti-mouse labeled with FITC secondary antibody. Then, all the samples were subjected to the capture of 3D pictures and the foci number was counted with confocal microscope. The FociPicker3D plugins of ImageJ was written and utilized to acquire information from the 3D confocal microscopic image of the IRIF in HeLa cells.

The following figures were analyzed for γ-H2Ax and 53BP1 IRIF kinetics in HeLa cells after application of 0.2 and 1.0 Gy X-rays. With immunofluorescence, the γ-H2AX IRIF number exhibited the similar behavior as 53BP1 in

the lower dose samples, while a rapid assemblage of γ-H2AX compared with the delayed kinetics of 53BP1 in 1.0 Gy irradiated cells (Fig. 1). We found more 53BP1 IRIFs in the lower dose samples at almost all the time points but the contrast measurement showed in the other one (Fig. 1). As illustrated in Fig. 1(b), the initial IRIFs formation in the higher dose samples was rapid compared with the control and lower dose samples (Fig. 1). The difference between 53BP1 and γ-H2AX IRIF dynamics was distinct at the time point range of 5 to 35 min (Fig. 1(b)), after that a slow decay occurred in both samples (Fig. 1). To determine the fine recruitment kinetics of both proteins in cell line HeLa, We analyzed the total fluorescence intensity of both proteins in HeLa cells. With the immunofluorescence assay as sated above, the total intensity was utilized as an index to investigate the finer behavior of IRIFs than the number of foci. Indeed, the tendency of curves in Fig. 2(a) agreed with kinetics of IRIFs in Fig. 1(a), although the absolute difference is significant. However, the maximum of proteins intensity presented after irradiation time 35 min, especially 53BP1 (Fig. 2(b)), which were not closely in line with the number of foci (Fig. 1(b)). At the end of time course, there were obvious differences between experimental samples and the control (Fig. 2(b)). The results showed that the recruitment kinetics were not synchronous with those in the formation of IRIFs in the upper doses amples. There might be some delayed action in the assemblage process. These evidences could suggest the resistant DSBs induced by ionization were more complex than the most one.

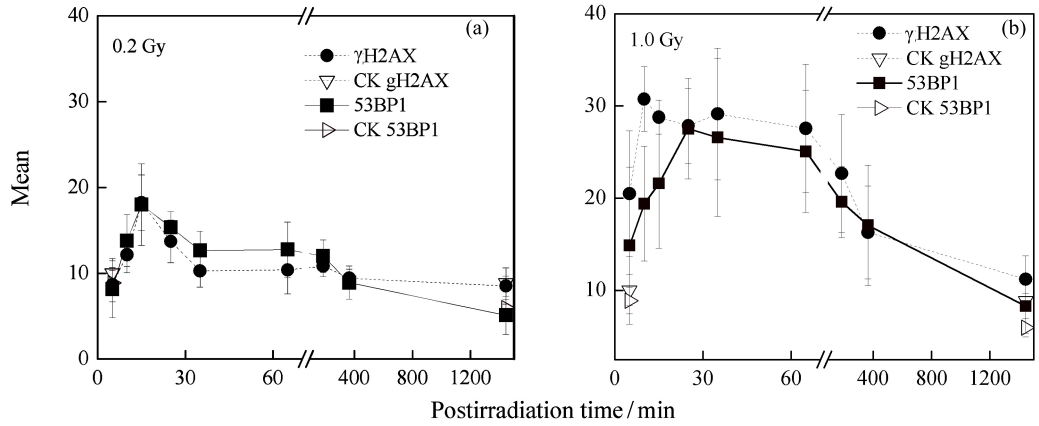

Fig. 1 The kinetics of γ-H2AX and 53BP1 foci number per cell. More than 1 000 cells were processed by FociPicker3D.

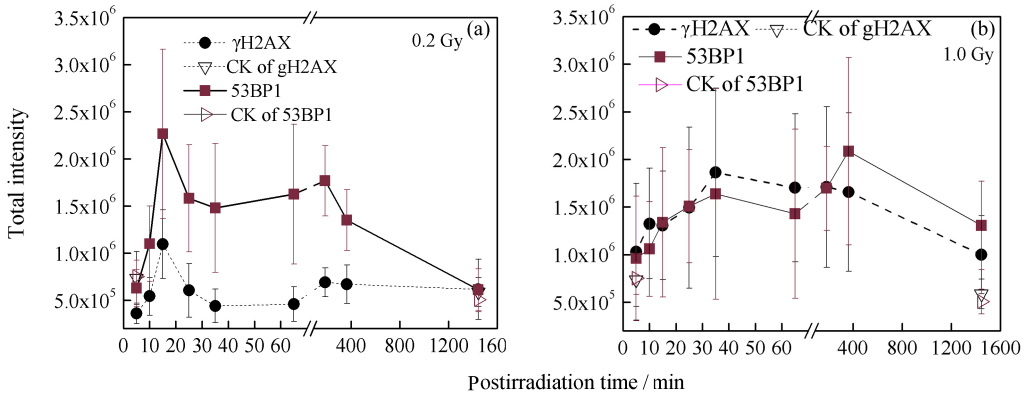

Fig. 2 The kinetics of γ-H2AX and 53BP1quantity per cell, total intensity can be regarded as protein quantity approximately in immunofluorescenceassay.

The assembly of proteins at DSBs site occurs in a highly ordered, strictly hierarchical and rapidly fashion. The quantification of γ-H2AX and 53BP1 foci allows the estimation of DNA repair and response. This work has been attributed to the DNA damage and repair induced by low dose X-rays. The application of fine revaluation index contributs to precise assessment.

References

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