STED NANOSCOPY REVEALS CORRELATION OF DNA REPAIR FACTORS Rad51 and BRCA1 AT α-PARTICLE INDUCED DSB.

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Ionizing radiation is a permanent threat to the human body. Our DNA is an especially prominent target for ionization events and these often result in fatal DNA damage lesions such as DNA double-strand breaks (DSB). DSB can lead to cell death or mutations and consequent cancer development. Over time, cells have developed several specific DNA damage repair pathways. The investigation of DSB repair mechanisms therefore helps to understand cancer development and forms a base for improvements in medical cancer treatment.In this study, the involvement of DSB repair proteins Rad51 and BRCA1 are characterized during homologous recombination (HR) of α-particle irradiation induced DSB. HR uses the homologous sister chromatid as a template to rejoin the loose DNA ends. Following a DSB induction, repair proteins cluster in so called ionizing radiation induced foci (IRIF) at the damage site. Structural analysis of these damage induced protein complexes requires microscopy methods beyond the diffraction barrier of 200 nm. To overcome this diffraction limit, STED nanoscopy with a lateral resolution of 105 nm was used. In combination with a protein correlation analysis method based on the rPDM published by Reindl et al. 2015 [1], a significant pattern in repair involvement of Rad51 and BRCA1 was discovered over a timeframe of 0-24 h repair time. IRIF containing both proteins are visible as early as 13 min after irradiation. In this early stage of homologous recombination the correlation has a maximum of 93% correlating regions which decays to 58% after 2h of repair. At 4h post irradiation, correlation returns to 92% only to decrease again in the late stages of repair (up to 24h) to 80%. This time dependent correlation during repair shows that the functions of BRCA1 and Rad51 vary throughout damage response and homologous recombination. The resulting data of the correlation analysis of BRCA1 and Rad51 also imply a subdivision of BRCA1 and Rad51 interaction into 3 phases during HR (early stage, processing stage, late stage). Furthermore Intensity plots measuring the localization of BRCA1 and Rad51 show local exclusion within the IRIF and therefore support predictions of non-existing direct protein interaction. [2]

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