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DNA-PK: A Perpetrator in Disguise

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Cancer-causing mutations are caused by genotoxic agents from various sources such as chemicals in environmental pollutants, cigarette smoke, excessive consumption of alcohol, and excessive sunlight exposure as well as exposure to ionizing radiations such as X-rays, γ-rays, alpha particles, to name a few [1]. In addition to this, cancer chemotherapy and radiation therapy are a major source of DNA damage and hence a possible cause of cancer or cancer relapse. The occurrence of mutations following genotoxic insults is often attributed to the faulty or futile repair of damaged lesions.

Innumerable studies pertaining to the genesis of cancer have focused on mutated genes. However, there are very few reports which have studied mechanism(s) of tumor induction in the perspective of the DNA-damage caused by genotoxic agents. Paules group showed that "cancer-predisposition programs" were induced in lymphocytes following DNA damage [2]. Furthermore, Fung and colleagues hypothesized that DNA damage induced following apoptosis induction remains unrepaired and possibly results in oncogenic transformation [3]. Yang et al. through their experiments exhibited the ability of heavy ions to induce DNA damage leading to transformation, however the mechanism remained an open question [4].

With regards to in vivo models, although DNA-damaging agents such as N-methyl-N-nitrosourea (MNU) [5] or 7,12-dimethylbenz[a]anthracene (DMBA) [6] have been widely used to induce mammary tumors in rodent models, the exact mechanism of tumor induction remains elusive. MNU induced rat mammary tumor is one of the widely used model to successfully screen therapeutics as well as to answer intriguing questions related to tumor progression. However, the mechanism of tumor induction by MNU is debateable The initial hypothesis was that MNU induces a point mutation at the 12th codon of H-ras gene [7]. The frequency of the H-ras mutations was also showed to reduce upon increasing doses of MNU albeit increase in tumor yield [8]. Additionally, it was shown that not all the MNU-induced mammary tumors harbor this mutation [9-14]. Furthermore, Cha et al. [10] and Maffini et al. [11] also demonstrated that H-ras mutations were also present in the untreated animals. Besides these, Maffinin et al. also showed that stroma was the primary target of MNU and the treatment of stroma alone was sufficient to induce tumor formation [11]. This report also claimed that tumor formation was independent of the H-ras mutation initially identified by Zarbl et al. [7]

Given the extent of exposure to DNA damaging agents in everyday life, and lack of knowledge about the precise mechanism of tumorigenesis following DNA damage; the study of the mechanism of DNA damage induced transformation becomes imperative. In a recent study, using the 3-dimensional breast acinar model, we demonstrated that alkylation damage induced cellular transformation, which was mediated via the activation of DNA-PK, a protein involved in the DNA repair process [15]. DNA-PK has been demonstrated in various studies to have diverse functions [16]. The significant role played by this complex is in the NHEJ pathway of DNA damage response (DDR) following the formation of DSBs. DNA-PK is a holoenzyme made up of two regulatory subunits Ku70 and Ku80 as well as a catalytic subunit, DNA-PKcs, responsible for the kinase activity [17,18]. Known as a critical player in safeguarding the genome, DNA-PK has been reported to regulate the entry into mitosis depending on the presence or absence of DNA damage, thus contributing to genomic stability [19]. Supporting its role as a guardian of the genome, Morozov et al., reported that inhibition of DNA-PKcs resulted in increased sensitivity to genotoxic agents, whereas somatic mutations in DNA-PKcs, the catalytic subunit of DNA-PK, contributes to genomic instability by interfering with repair of double strand breaks [20]. Furthermore, inhibition of the catalytic subunit was reported to cause chromosome mis-alignment as well as interfere with spindle formation [21]. DNA-PK has also been shown to play a vital role in cancer cells developing radio-resistance eventually resulting in the cells exposed to fractionated-radiation for a long period of time repairing any DNA damage incurred, faster than the unexposed cells [22]. Taken together, these studies suggest the protective role played by DNA-PK in safeguarding the genome.

On the contrary, the interaction of DNA-PK with Snail1 is known to promote chemo-resistance as well as genomic instability. [23,24]. Besides its well-established role in DDR, DNA-PK has been implicated to play a role in various cancers. However, the exact role of DNA-PK in cancer is ambiguous. PRKDC, the gene encoding for DNA-PKcs, has been found to have low expression in lung cancers [25], ovarian cancers [26] as well as gastric cancer [27]. On the other hand, a higher expression has been reported in hepatocellular carcinoma [28], prostate cancer [29] and melanomas [30]. Recent reports suggest over-expression of DNA-PKcs regulates metastasis of prostate cancer cells through transcriptional regulation [29]. Furthermore, DNA-PK inhibition was found to inhibit the formation of primary melanoma, delayed metastasis to lymph nodes as well as inhibited secretion of MMPs [30]. However, its role in breast cancer remains elusive. Cimino et al. identified PRKDC as one of the genes being upregulated in breast cancer patients which were also found to be associated with reduced survival [31]. This result was in concordance with the data collected by van de Vijver et al. [32] and Sotiriou et al. [33]. Our study illustrates for the first time the role of DNA-PK in breast tumorigenesis [15]. In our study, MNU was used to induce DNA-damage, which in turn resulted in activation of DNA-PKcs which eventually resulted in transformation of non-tumorigenic breast epithelial cells. DNA-PKcs, which gets activated following DNA-Damage primarily to recruit the repair machinery, was found to be able to alter various characteristics of the cell. Our study also suggested that DNA-damage induced phosphorylation of DNA-PKcs resulted in its constitutive activation,

since inhibition of DNA-PK at a later time point was able to reverse the transformation phenotypes induced. The autophosphorylation tendency of DNA-PK can explain this speculation. In addition to this, loss of basal polarity has been found to reduce the efficiency of repair of DSBs [34] which in turn would either result in constitutive activation of DNA-PK or result in accumulation of chromosomal aberration when these cells enter cell-cycle and thus lead to carcinogenesis.

One of the striking features in cells that were exposed to MNU was their altered Golgi morphology. The intracellular trafficking function of such disrupted Golgi was found to be impaired. This Golgi phenotype was in corroboration with the report by Farber-Katz et al. [35], wherein the dispersed Golgi phenotype was attributed to the activation of DNA-PK. The dispersal was attributed to the phosphorylation of GOLPH3 induced by activated DNA-PK. Epithelial cells are characterised by their ability to polarize. Investigation of the effect of methylation damage in the establishment of polarity, we observed disruption of apical as well as basolateral polarity. Accumulation of laminin V, as well as integrin-α3 in the cytoplasm coupled with alteration of composition of the cell membranes observed, can be attributed to the impaired trafficking. Laminin V phenotype observed has also been referred in literature to be indicative of invasive phenotype. Invasive cells are known to break down the basement membrane to invade the nearby tissue and disseminate to distant organs [36]. Apart from this, down-regulation of this α6-integrin subunit has been observed in cells metastasizing to the pleural cavity and parenchyma [37]. Loss of laminin V and α6-integrin from the basal region in acinar structures, enhanced secretion of MMP-9 and ability to cleave collagen observed in our study corroborated with this observation and suggested the induction of invasiveness in cells following DNA damage. Epithelial–mesenchymal transition (EMT) which is one of the initial processes in invasion was also found to be induced in the MNU-treated cells. These cells also gained the ability to survive under anchorage independent conditions, which is considered the most stringent criteria to identify transformed cells. The different phenotypes namely EMT, invasion and anchorage independence besides altered Golgi phenotype, induced by DNA damage was reversed following inhibition of DNA-PK, thus confirming the central role played by DNA-PK in methylation damage induced transformation. The interaction between Snail1 and DNA-PK has been reported to result in increased activity of Snail1 [24]. Increased Snail1 activity results in EMT, which confers to the results of our study where upregulation of Snail1 was observed. However, surprisingly DNA-PK inhibition was unable to restore the impaired intracellular trafficking suggesting that DNA-PK induced transformation was independent of its effect on Golgi trafficking.

SJ Field and group in their study have illustrated that DNA damage by various agents including radiation, induced dispersal of Golgi [35]. Further NEU and MNU result in different kind of mutations where NEU is known to induce random mutations [38] while MNU induces mutations at specific sites defined by a particular consensus sequence [39]. The ability of NEU to induce transformation [40] with phenotypes similar to that induced by MNU indicates that the effect caused by methylation damage is due to DNA damage induced in general and not specific to the chemical used. Given that DNA damage is a generalized effect, activation of alternate pathways cannot be negated. However, reversal of phenotypes induced following exposure to MNU by DNA-PK inhibition suggests the central and novel role of DNA-PK in breast tumorigenesis. Taken together, the study highlights the vast range of effects which can be induced in cells by DNA-damage *via* activation of DNA-PK.

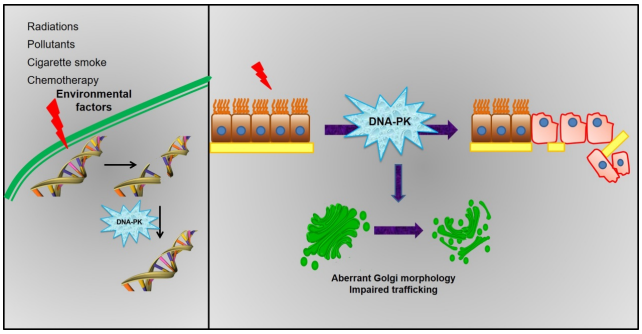

Figure 1: Schematic showing the mechanism of MNU-induced transformation. DNA-PK, one of the key proteins involved in DDR pathway, repairs DNA damage caused by various environmental factors. However, at the cellular level, MNU induced activation of DNA-PKcs, not only altered Golgi morphology and impaired intracellular trafficking to the plasma membrane but also resulted in transformation of breast epithelial cells.

Conclusion

DNA damage, if left unrepaired can lead to nuclear effects which might result in genomic instability. This paradigm of effect of DNA damage has been well characterised. However, little is known till date, about the effects of DNA damage on cell apart from the nucleus. Our study highlights the consequence of DNA damage and provides mechanistic insights into the process. It illustrates the novel role played by DNA-PK in the process of transformation. It demonstrates the irony that the surveillance mechanism (activation of DNA-PK) in the process of safeguarding the genome, itself triggers the process of transformation. This fact can be exploited to improve cancer therapy. Small molecule inhibitors of DNA-PK have been developed with the aim to improve chemo as well as radio sensitivity and a few of these compounds have entered clinical trials [41]. Use of an inhibitor to DNA-PK as an adjuvant to chemotherapy can prove to be beneficial not only to improve sensitivity to cancer therapeutics but also prevent such inadvertent effects. Nevertheless, there could be multiple pathways which may synergistically contribute to the transformation apart from the mechanism summarized in the Figure 1. This calls for further interrogation into the process and explore the other pathways to aid in identifying the various molecular players. The information thus gathered can then be used to design novel therapeutic strategies or modify the present strategies. The model established in our study can be further exploited to study alternate pathways as well as used for therapeutics screening. In addition to this, such a model can also be used to understand how early transformation occurs and identify the initial set of gene(s) that get deregulated in the process of transformation. Understanding this phenomena and recognition of key candidate genes as biomarkers could further help to design novel or modify available therapeutic strategies.

Conflicts of Interest

The authors disclose no potential conflicts of interest.

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