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Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes

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Abstract

The mechanism by which radiation-induced genomic instability is initiated, propagated and effected is currently under intense scrutiny. We have investigated the potential role of altered genomic methylation patterns in the cellular response to irradiation and have found evidence for widespread dysregulation of CpG methylation persisting up to 20 population doublings post-irradiation. Similar effects are seen with cells treated with medium from irradiated cells (the 'bystander effect') rather than subjected to direct irradiation.

Using an arbitrarily primed methylation sensitive PCR screening method we have demonstrated that irradiation causes reproducible alterations in the methylation profile of a human keratinocyte cell line, HPV-G, and have further characterised one of these sequences as being a member of a retrotransposon element derived sequence family on chromosome 7; MLT1A. Multiple changes were also detected in the screen, which indicate that although the response of cells is predominantly hypermethylation, specific hypomethylation occurs as well. Sequence specific changes are also reported in the methylation of the pericentromeric *SAT2* satellite sequence. This is the first demonstration that irradiation results in the induction of heritable methylation changes in mammalian cells, and provides a link between the various non-radiological instigators of genomic instability, the perpetuation of the unstable state and several of its manifestations.

Keywords: DNA methylation; Human keratinocytes; Genomic instability

1. Introduction

The paradigm in which the deleterious effects of ionising radiation are attributed to chemical alteration of DNA through linear energy transfer is well established. However, accumulating evidence suggests that other phenomena may also occur when cells are irradiated which establish a heritable phenotype of genomic instability (radiation-induced genomic instability: RIGI). The manifestation of this instability may be delayed for many cell divisions, it will then show itself in a variety of ways depending on the cell system used. Delayed lethal mutations (delayed reproductive cell death), delayed apoptosis, non-clonal cytogenetically detectable chro-

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mosome aberrations, often involving chromatid lesions, minisatellite instability, micronucleus formation and, potentially, transformation, are all well documented endpoints [1–5]. The delayed nature of onset may be of particular relevance to neoplasia, but the relationship is still unclear.

The phenomenon of delayed response to radiation is puzzling for several reasons. Firstly the very high frequency and non-clonal nature of the lesions seen, and the delay in their expression, are not consistent with a classical mutagenesis mechanism, where lesions would be expected to manifest themselves at the time of irradiation, then, if viable, to propagate clonally. Secondly the delayed onset of many endpoints is not easily explained by current theory except as the formation of a 'mutator phenotype' involving for example, a repair or damage sensing gene mutation, although even here the non-clonality and the very high frequency of the effect (seen in almost all surviving descendents) argue against this explanation. A simple relationship between lethal chromosomal aberrations and reproductive cell death has recently been excluded in at least one system [6].

Attempts to establish if there are consistent patterns of gene dysregulation in response to irradiation have demonstrated the expected up or down regulation of gene products associated with immediate, post-irradiation, DNA repair processes [7], yet few of these changes persist through the tens of generations during which instability occurs in some cell types [6,8,9]. To explain this, non-gene dependent processes such as persistent changes in intracellular redox potential have been postulated [9,10], however, the mechanism whereby this could result in genomic instability and delayed reproductive cell death is still unknown. Previous studies in intact plants, mice and CHO cells have indicated alterations in global methyl cytosine content following irradiation [11], but Kovalchuk et al. [12] have recently reported gene specific and tissue specific alterations in methylation in response to both acute and chronic low dose irradiation in intact mice.

DNA methylation in mammalian cells is effectively restricted to the sequence CpG and methylcytosine is widespread in the genome. It is found predominantly in intermediate repetitive sequences [13], but is also present in the CpG islands at the 5′ ends and within transcription units, involved in their regulation. In general CpG hypermethylation is associated with transcriptional repression, although in some systems, particularly those subject to regulation by genomic imprinting, the reverse may be the case. However, the abnormal modification of the CpG methylation profile of cells is generally deleterious and has pathological consequences [14].

1.1. DNA methylation and genomic instability

In humans dysregulation of methylation, usually as a consequence of aberrant or absent activity of the DNA methyltransferases (DNMTs), has been implicated in carcinogenesis. Important targets affected in this process include promoter specific methylation of tumour suppressor genes such as the DNA repair enzyme *MLH1* [15,16] or *RASSF1A* [17,18] or regulators of proliferation such as CDKN2A [19]. While it is clear that alterations in the expression of DNA repair associated enzymes, such as *MLH1* may affect mutation rates and genomic stability through well characterised processes, instability has also been associated with altered methylation of non-coding and retroposon related sequences, for example, in microsatellite instability in colorectal cancer, retroviral activation and elevation of global mutation rates [20–24]. Region specific hypomethylation of pericentromeric satellite sequences has previously been reported in human ICF syndrome (immunodeficiency–centromeric instability–facial anomalies), which results from deficiency in DNMT3B activity [25]. This is associated with decondensation of heterochromatin and extensive non-clonal pericentromeric rearrangements of specific chromosomes consisting of whole-arm deletions, chromosome breaks, multiradials, and translocations, reminiscent of the genomic instability seen in response to irradiation. Once established, methylation patterns are reproduced during DNA replication as hemi-methylated DNA is the preferred substrate for the maintenance methylase, DNMT1, which faithfully reproduces the methylation pattern on the new DNA strand. Thus once modified it might be expected that the aberrant pattern would be faithfully inherited at each cell division. From the existing available data, therefore, alteration of methylation is an attractive mechanism for both the propagation of genomic instability following irradiation and for the underlying molecular lesion.

Using an arbitrarily primed methylation sensitive PCR screening technique we demonstrate here that irradiation causes stable long term changes in the methylation profile of a human keratinocyte cell line and have gone on to characterise one of these sequences as being a copy of an LTR related retrotransposon family on chromosome 7. Multiple changes were also detected in the screen, which indicate that although the response of cells is predominantly hypermethylation, specific hypomethylation is seen as well.

We suggest that global dysregulation of genomic methylation may be a mechanism by which RIGI is propagated and may contribute to some or all of the endpoints seen in response to cell irradiation.