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Genomic instability in rat: Breakpoints induced by ionising radiation and interstitial telomeric-like sequences

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Abstract

The Norwegian rat (*Rattus norvegicus*) is the most widely studied experimental species in biomedical research although little is known about its chromosomal structure. The characterisation of possible unstable regions of the karyotype of this species would contribute to the better understanding of its genomic architecture. The cytogenetic effects of ionising radiation have been widely used for the study of genomic instability, and the importance of interstitial telomeric-like sequences (ITSs) in instability of the genome has also been reported in previous studies in vertebrates.

In order to describe the unstable chromosomal regions of *R. norvegicus*, the distribution of breakpoints induced by X-irradiation and ITSs in its karyotype were analysed in this work. For the X-irradiation analysis, 52 foetuses (from 14 irradiated rats) were studied, 4803 metaphases were analysed, and a total of 456 breakpoints induced by X-rays were detected, located in 114 chromosomal bands, with 25 of them significantly affected by X-irradiation (hot spots). For the analysis of ITSs, three foetuses (from three rats) were studied, 305 metaphases were analysed and 121 ITSs were detected, widely distributed in the karyotype of this species. Seventy-six percent of all hot spots analysed in this study were co-localised with ITSs.

Keywords: Interstitial telomeric-like sequences; X-irradiation; Genomic instability; Rat

1. Introduction

The Norwegian rat (*Rattus norvegicus*, RNO) is the most widely used experimental species in biomedical research and it has also become a model organism for many multifactorial human disorders. Despite its importance in research and the achieving of the whole genome sequencing of the rat, little is known about its genomic architecture. In fact, its chromosomal function involves very complex interactions that go further than what can be understood about primary sequences [1]. Conse-

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quently, stability of a genome does depend not only on its genetic sequence, but rather also on its own genomic architecture. Thus, the study of the genomic architecture, from the instability point of view, would also contribute to a better understanding of the chromosomes of rat.

Ionising radiations are physical agents that can induce mutations [2] in all stages of the cell cycle. Double-strand breaks (DSBs) are the primary lesions of DNA that allow a direct formation of structural or numeric chromosomal aberrations after defective repairing and these genotoxic effects in cells can be perceived at the cytogenetic level. The cytogenetic effects of ionising radiations in cells have been widely used for the study of genomic instability. Previous cytogenetic studies in Primates have suggested the existence of chromosomal regions more prone to break after treatment with physical and chemical agents [3–7], and also other studies in vertebrates have shown the importance of interstitial telomeric-like sequences (ITSs) in chromosomal instability and evolutionary processes [8–10]. Indeed, a correspondence has already been reported among breakpoints by ionising radiation, ITSs and fragile sites (FSs), proto-oncogens, breakpoints involved in chromosomal cancer rearrangements and evolutionary breakpoints [3,7].

ITSs consist of telomeric-like $(TTAGGG)_n$ tandem repeats located at non-terminal regions in eukaryotic chromosomes and their pattern in vertebrates can vary among closely related species [11]. They have been classified into different types according to their organisation: short, subtelomeric and fusion ITSs and large blocks of these sequences [8,12,13]. Short ITSs are made up of short sequences of TTAGGG units, essentially exact and arranged in the same orientation. They are polymorphic and highly unstable [14]. Subtelomeric ITSs are also polymorphic and unstable sequences, but they consist of some hundreds of base pairs, including some degenerated units in the same orientation [14]. They have been observed to be associated with telomeric proteins and can even contain genes [15]. Fusion ITSs contain two head-to-head blocks of TTAGGG flanked by subtelomeric sequences, whereas large blocks of telomeric sequences are found in chromosomal pericentromeric regions in different non-human species [11,12].

Previous studies performed in mammals, including humans, have postulated that some ITSs can be considered as a result of ancestral chromosomal reorganisations [9,16] and of intrachromosomal reorganisations [17,18]. Some of them are associated with human disorders that have caused an internalisation of telomeres [1,19,20]. In fact, Ijdo et al. [16] identified two inverted DNA sequences of telomeric DNA in human chromosomal band 2q13, considered a relic of an ancient telomeric-telomeric fusion of two ancestral primate chromosomes. It has also been postulated that they could be unstable sequences where fissions and internal reorganisations could occur during the evolution of the karyotype, the storage of new telomeres or fission points being where chromosomal reorganisations can be fixed during the evolutionary process [9,21]. Recent studies have postulated that a large amount of ITSs have been generated by the insertion of telomeric sequences in repairing DSBs, so, ITSs could simply mark breaksites within unstable regions [8,10,13].

Numerous authors coincide in the existence of a relationship between ITSs and events of chromosomal instability, such as recombination, amplification and even retro-transposition, in bacteria, yeast, Chinese hamster [22–25], other rodent species [26] and also in the human karyotype [10]. There are remarkable results that give evidence that ITSs are preferential sites to solve chromosomal aberrations and that these sites show both spontaneous instability [27,28] and induced instability [29,30]. In particular, radiosensitivity of ITSs has already been demonstrated [31,32]. Moreover, a co-localisation of ITSs and FSs has been found at the cytogenetic level in rodents and primates [9,27,33–35], and previous cytogenetic studies in non-human primates have indicated that there is a relationship among evolutionary breakpoints, FSs and the existence of ITSs [7,9].

The main objective of this work is to characterise unstable regions in the *R. norvegicus* genome so as to elucidate the genomic architecture of this species. In order to study the architecture of the rat karyotype relating to its chromosomal instability, the chromosomes and chromosomal bands involved in ionising radiation effects and those containing ITSs are described.

2. Materials and methods

2.1. Treatment of animals and obtainment of foetuses

A total of 17 (14 for the detection of breakpoints by X-rays and three for the detection of ITSs) *R. norvegicus* female Sprague–Dawley rats ($2n = 42$, Servicio de Animales de Laboratorio, Universidad de Murcia and Servei d'Estabulari, Universitat Autònoma de Barcelona, Spain) were mated with males of known fertility for two weeks. After 14–16 days of pregnancy, the pregnant females were sacrificed in order to obtain foetal tissues.

2.2. Cell cultures and chromosome preparations

A total of 52 foetuses were analysed (49 for the detection of breakpoints by X-rays and three for the detection of ITSs).