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Metal ion interactions with the minisatellite DNA of H-ras oncogene.

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Abstract

Over the years there has been extensive research on the molecular interaction of minisatellite DNA with the human gene Ras and the impact of these interactions in regulating transcription of the oncogene. Metal ions such as Hg(II), Cd(II), and Pb(II) are known to act at the cellular level and interact with biomolecules both in the cytoplasm and the nucleus, leading to changes in the activity of key biomolecules in biochemical processes and affecting the integrity of the cell. What is not known and has attracted considerable interest is the interaction of such metallotoxins with minisatellite DNA and the ensuing induction on the H-Ras activity. To this end, the effect of biotoxic metal ions on the transcriptional regulation of minisatellite DNA on the H-Ras activity constitutes the crux of the present investigation.

Keywords: H-ras, metal ion toxicity, minisatellite, DNA damage, transcription

1. Introduction

In the past decade there has been extensive research on the molecular interactions of minisatellite DNA with the human gene Ras and the impact of these interactions in regulating transcription of the oncogene [1]. The *H-ras* oncogene has an associated downstream minisatellite that is believed to be capable of influencing *H-ras1* gene transcription. Rare alleles of this minisatellite have been associated with a predisposition to acute leukemia and carcinomas of the lung, testis, breast, colorectum, urinary bladder and non-Hodgkin's lymphoma. The *H-ras* minisatellite is located approximately 1 kilobase (kb) downstream from the *H-RAS* gene. This polymorphism, which has a variable number of tandem repeats, *(H-ras1* VNTR) consists of a 28 base pair (bp) consensus sequence [2-5]. Changes in the number of these repeat units permit detection of the polymorphisms by restriction enzyme digestion and Southern analysis.

Thirty alleles of approximately 1000 to 3000 bp have already been described at this locus. However, four common alleles represent 94% of all alleles in whites [2]. The rare alleles occur at frequencies between 0.2-1.3%. In addition to length variability, most previously sequenced human minisatellites were also shown to differ in the interspersion pattern of variant repeat units along alleles. The *H-ras* minisatellite sequence (GenBank Accesion Number 500277) has two positions (7th and 15th in the 28 bp repeat,) where variants (G or C) occur. These sequence variants could be detected by the minisatellite variant repeat (MVR) analysis. In fact, a recent paper [5] reports the MVR sequences of common and rare alleles over the first 20 repeats at the Send.

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In many cases, these metal-biochemical actions are not known, even though in some cases such actions target specific biological pathways. In the case of many cancers (breast cancer, lung, prostate), oncogenes play an important role, getting involved in biochemical pathways leading to neoplasias. A representative such oncogene is the H-Ras oncogene. The discovery that DNA minisatellite downstream of the H-Ras gene plays an important role in the transcriptional regulation of this oncogene at the cellular level presents a challenge in the exploration of toxic metal-H-Ras interactions and carcinogenicity. Lethal alleles of minisatellite DNA were found at increased frequencies in patients with breast cancer. Since toxic metal ions interact with gene targets and the aforementioned metal ions are known carcinogens, it is imperative to investigate the effect of these toxic metal ions in processes involving specific oncogene targets and leading to carcinogenesis. The interaction of hazardous metal ions such as Hg(II), Cd(II), Ni(II), Pb(II) can cause DNA damage and permanent changes in gene expression, with subsequent development of neoplasias [1]. It is worth pondering over that such metal ion interactions with the genetic material are an imminent danger to life and thus requires extensive research to comprehend and delineate. It is necessary to gain knowledge on the subject, and not only for the treatment of serious illnesses resulting from such causes, while concurrently looking into advanced technologies for the prevention of cancer and protection of cellular physiology in humans.

2. Materials and methods

The H-ras alleles a0.26, a1, α2.1, a2.3, a2.4 and a3 were isolated using RFLP techniques. They were subsequently used for the creation of chimeric plasmids containing the H-ras oncogene.

3. Results and Discussion

The genetic analysis of the variable tandem repeat polymorphisms at the H-ras-1 gene locus showed that the H-ras-1 gene is located on human chromosome 11p15.5. There are two VTR polymorphisms. Between the pseudo exon (0) and exon 1 there is a triallelic 6 bp repeat motif that is reiterated two, three, or four times [4]. The longest and shortest allele types are indicated. Approximately 1.4 kb to the 3′ end of the structural gene there is a multiallelic VTR consisting of a 28 bp consensus sequence that is reiterated between 30 and 110 times.

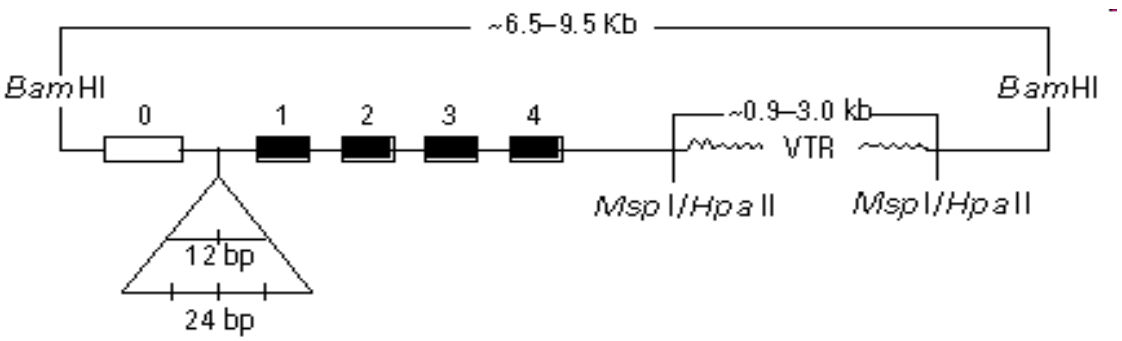

Figure 1. The VNTR locus of H-ras gene.

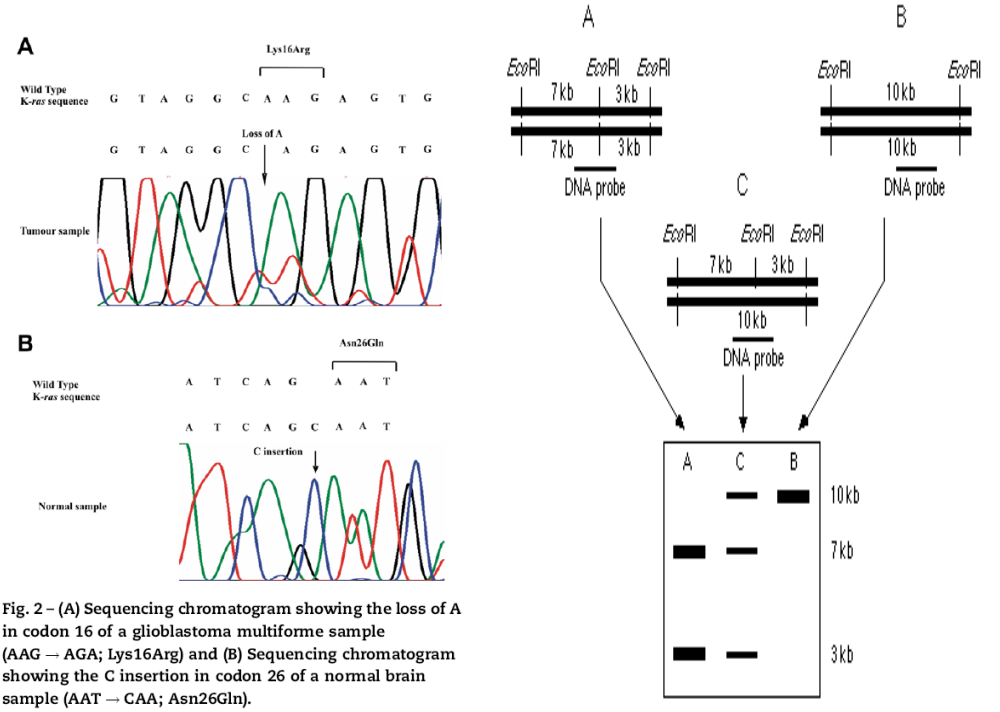

Fig. 2 – (A) Sequencing chromatogram showing the loss of A in codon 16 of a glioblastoma multiforme sample (AAG → AGA; Lys16Arg) and (B) Sequencing chromatogram showing the C insertion in codon 26 of a normal brain sample (AAT → CAA; Asn26Gln).

Figure 2A, 2B (Left). DNA sequencing is used to detect possible mutations in the DNA molecule, ***Figure 2C (Right)***. RFLP is a variation in the DNA sequence of a genome that can be detected by breaking the DNA into pieces with restriction enzymes and analyzing the size of the resulting fragments through gel electrophoresis

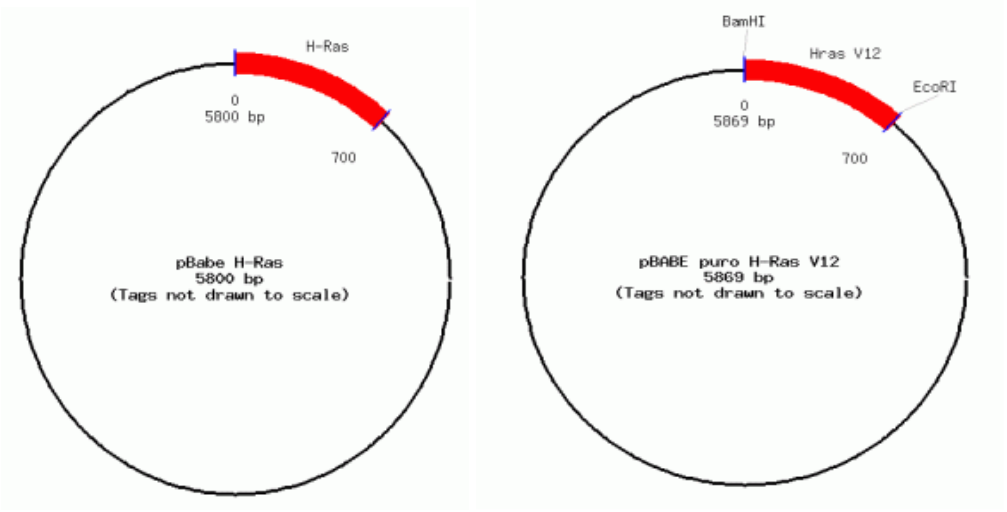

Figure 3. H-ras gene and the mutant H-ras gene G12V.

4. Conclusion

The discovery of direct and strong interaction between metal ions with the DNA, ushered in a massive research effort to fully understand and elucidate this interaction, aiming at the protection and treatment of the human body from such threats. The human contact with metal ions of heavy elements, is likely to cause its own pathologies, including carcinogenesis [4,5]. The interaction of hazardous metal ions such as Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} can cause DNA damage and permanent difference in gene expression, with subsequent development of neoplasias.

Such metal ion interactions with the genetic material is an imminent danger to life and thus requires extensive research in this area. It is necessary to establish knowledge of the subject, not only for treatment of serious illnesses resulting from such causes, but also for the prevention of carcinogenic events and protection of human physiology.

The work carried out so far exemplifies the significance of discrete and well-characterized plasmids a) carrying all possible alleles, and b) being capable of interactions with the heavy toxic metal ions.

Ongoing work in the lab targets the arising binary interactions and looks into the transcriptional regulation of the upstream HRAS gene involved in tumorigenic processes.

Acknowledgements

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