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The association of carcinogenic metal ions with the minisatellite DNA of H-ras and the impact on the transcription mechanism

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Abstract

In the past years there has been extensive research on the molecular toxicity of metal ions and the influence that metal ions exert on the genetic machinery of human cells. 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 molecules in biochemical processes and affecting the integrity of the cell. The interaction of such metal ions with oncogenes such as H-Ras and it downstream minisatellite DNA constitutes the crux of the present investigation. The influence of biotoxic metal ions on the genomic machinery sets the stage for the development of new knowledge that will aid in the development of diagnostic and therapeutic approaches in cancer.

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

1. Introduction

Over the past years there has been extensive research on the molecular toxicity of metal ions and the influence that metal ions exert on the genetic machinery of human cells. 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 molecules in biochemical processes and affecting the integrity of the cell. In many cases, these metal-biochemical interactions are not known, even though in some cases such interactions are focused on 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 biotoxic 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, ultimately 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.

The *H-ras* oncogene has an associated minisatellite that is believed to be capable of influencing *HRASI* gene transcription. Rare alleles of this minisatellite have been associated with a predisposition to acute

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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, *(HRASI* VNTR) consists of a 28 base pair (bp) consensus sequence. 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 [2,3]. The rare alleles occur at frequencies between 0.2-1.3%. In addition to length variability, most previously sequenced human minisatellites also 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 [4].

In view of the paucity of knowledge in the specific field and the potential technological advances in the future, gaining knowledge on the subject may influence profoundly the therapeutic treatment(s) of serious illnesses resulting from such causes while concurrently set the stage for the development of advanced technologies toward prevention and protection of cellular physiology in humans. To this end, the present investigation aspires to delve into the developing interactions between biotoxic metal ions and ongogenic H-Ras/minisatellite DNA thereby inquiring into the influence over the transcriptional regulation of the oncogene related to the carcinogenic process [5].

2. Materials and Method

The H-ras alleles a0.26, a1, α2.1, a2.3, a2.4 and a3 were isolated using RFLP techniques. They were used subsequently for the generation of chimeric plasmids containing the H-ras oncogene. Isolation of the H-ras gene and the mutant H-ras gene G12V was also carried out. To achieve this goal, plasmids were acquired from Addgene (Cambridge, MA, USA) and using restriction endonucleases the specific genes were isolated. Moreover, different cell lines were used for the transformation of plasmids. Finally, further construction and characterization of plasmids that contain the specific alleles will ensue in several cell lines, which will be tested in the presence of various concentrations of toxic metal ions.

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 toward 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.

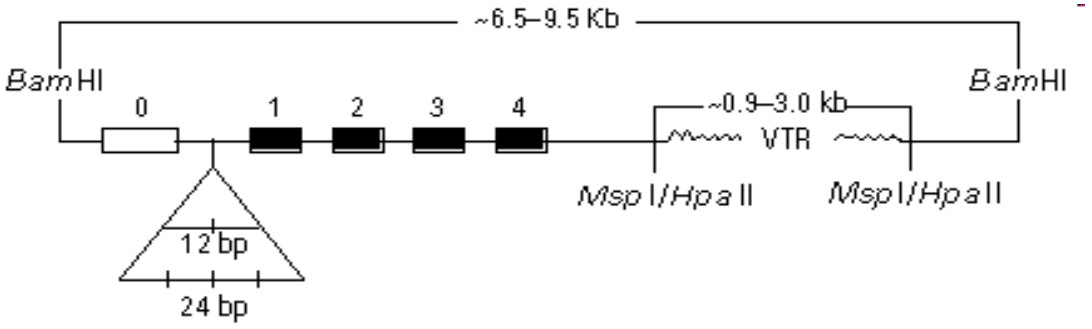

Figure 1. The VNTR locus of H-ras gene

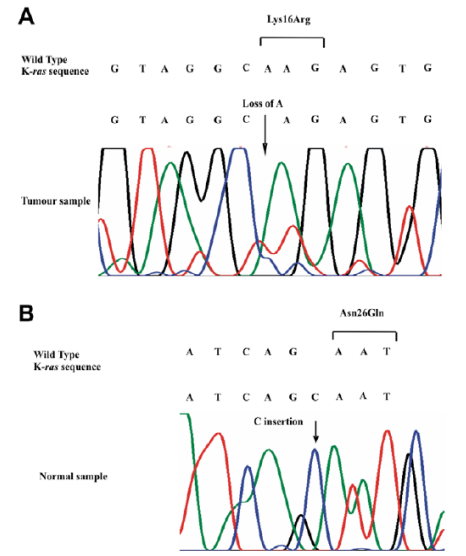

Fig. 2 – (A) Sequencing chromatogram showing the loss of A in codon 16 of a glioblastoma multiforme sample (AAG → AGA; Lys16Arg) and (B) Sequencing chromatogram showing the C insertion in codon 26 of a normal brain sample (AAT → CAA; Asn26Gln).

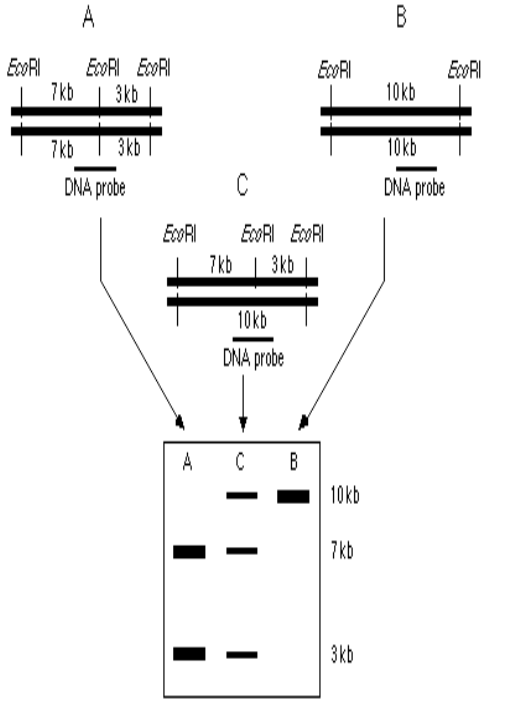

Figure 2A, 2B (Left) : DNA sequencing is used to detect possible mutations in the DNA molecule
Figure 2C (Right): RFLP is a variation in the DNA sequence of a genome that can be detected by fragmenting the DNA into pieces with restriction enzymes and analyzing the size of the resulting fragments by gel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

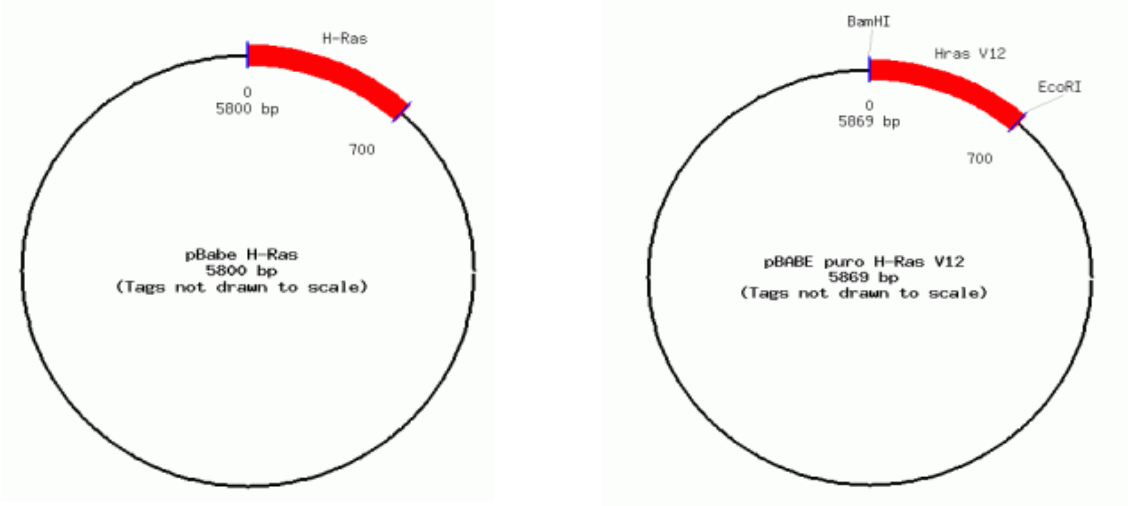

Figure 3. The H-ras and the mutant H-ras genes G12V were isolated. To achieve this goal, plasmids were purchased from Addgene (Cambridge, MA, USA) and these genes were isolated using restriction endonucleases

4. Conclusion

The discovery of direct and strong interaction between metal ions with DNA ushered in a massive research effort to fully understand and elucidate the mechanism of 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 specific pathologies, including carcinogenesis [4,5]. The interaction of hazardous metal ions such as Hg(II), Cd(II), Ni(II), Pb(II) 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 gain knowledge on the subject, not only for the development of treatments of serious illnesses resulting from such causes, but also for the prevention and protection of human physiology from threatening aberrant pathologies

The work carried out so far exemplifies the significance of discrete and well-characterized plasmids carrying all possible alleles and being capable of promoting interactions with heavy 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.

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