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HLA POLYMORPHISM IN CANCER

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ABSTRACT
HLA is the gene cluster presents in chromosome no 6 in human. The Polymorphism in this gene leads to many abnormality and diseased condition due to variation in the HLA antigens those are responsible for the activation of immunological molecules and cells. In our study total genome was isolated from Normal and diseased blood and was tested for the variation in HLA gene at its major allelic sites. HLA-SSP PCR was performed in which HLA allele specific primers were used. This analysis resulted in the alleles that can cause cancer. The alleles were expressed B0702 and It was found that theses allele is also responsible for causing leukemia. The normal blood is amplified by using the marker primer DRB301 as control.

KEYWORDS: Immunogenetics, HLA, MHC, molecular biology.

1. INTRODUCTION

HLA term indicates to the Human Leukocyte Antigen System, it's an important part of immunity. The genes responsible for controlling it are located on chromosome 6 on the short arm. It is also piece of hereditary area, called as the major Histocompatibility Complex (MHC) (Karan M.A. et al., 2002).

Genes of major histocompatibility complex that include gens of HLA are a major part of the immune response functioning. The HLA antigen, its primary role is self-identification and thus resistance to foreign bodies. the HLA sites, due to polymorphisms no individuals are identical so the population is ready to fend off or deal with the attack (Kirsten K et al., 2008). due to some antigens of HLA which are identified on most of the bodies tissues (instead of just blood cell) described antigens of HLA identification as "Tissue Typing" or "HLA Typing". According to the structural and functional provided by antigens, separated into two HLA classes, defined appropriately, Class I and HLA Class II. The total volume of whole of the Major histocompatibility is nearly 3.5 million bp (Fig.1).

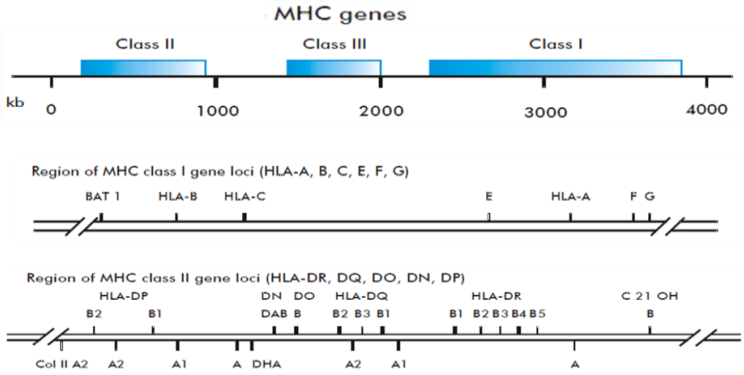


Fig. 1: Structure representing the overall size of MHC gene.

Inside of all that HLA Class I and HLA Class II genes each distributed Across around 33% of this length. The leftover area, often referred to as Class III, Locus containing loci in charge of supplementation, hormones, preparation of peptides intracellular and many formative qualities (Koike K et al., 2008).

In this way, the Class III region is not really a piece of the HLA complicated, But within the HLA locale it is situated, since its segments are mostly associated with HLA antigen elements or are undergo comparable monitor components to the HLA characteristics (Sanfilippo F, Amos DB 1986).

HLA Antigens Class I

HLA Class I antigens are called the cell surface glycopeptide antigens of the HLA-A, -B, and -C series (Butterfield L.H et al., 1999). The list of HLA Class-I antigens currently recognized is expressed on the surface of most of the body's nucleated cells. In addition, they are present in plasma in a soluble state and are adsorbed on the platelet surface. Depending on the precision, erythrocytes also adsorb HLA Class I antigens to different degrees. (e.g. HLA-B7, A28 and B57 are recognizable on erythrocytes as so called “Bg” antigens). Immunological findings show that the most important HLA Class I locus is HLA-B (which is also the most polymorphic), followed by HLA-A and then HLA-C. Also there are other loci in HLA Class I (e.g. HLAE, F, G, H, J, K and L), but most of these may not be relevant for "peptide presenters" as loci. HLA Class I antigens consist of a heavy chain 45 Kilodalton (KD) glycopeptide with three domains, non-covalently paired with b-2 microglobulin, which plays a significant role in sustaining the heavy chain structurally. Within the cell, the HLA Class I molecule is assembled and finally lies on the cell surface with a part incorporated into the cell membrane's lipid bilayer and has a short cytoplasmic tail (Fig. 2). Common design in general of the HLA molecules Class I, HLA molecules Class II and IgM indicates that the resemblance of the subunits is such that a shared relation between HLA and immunoglobulin is likely to return to certain primordial surface cell receptors.

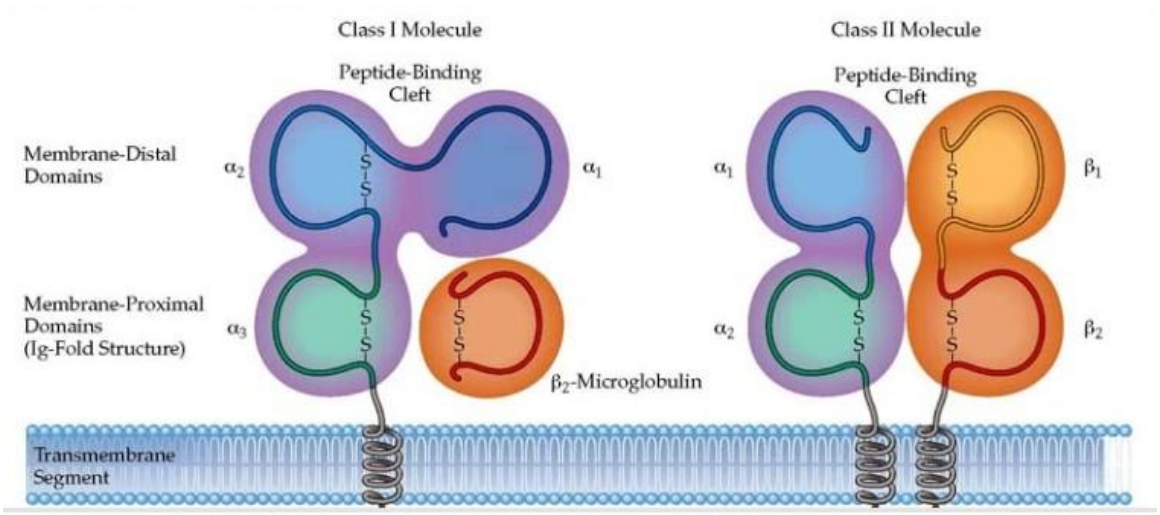


Fig. 2: Structure representing MHC –I and MHC-II Molecules.

HLA Antigens Class II

HLA Class II are the cell surface glycopeptide antigens of the HLA-DP, -DQ and -DR loci(Koike K et al., 2008). The delivery of HLA Class II antigens to the tissue is restricted to 'immune competent cells, including B-lymphocytes, macrophages, endothelial and activated T-lymphocytes. Class II HLA expression is induced by cytokines such as interferon g and in transplantation on cells that may not usually produce them; this is consistent with acute degradation of the graft. "The molecules of HLA Class II are composed of two chains each of them encoded on Chromosome 6 by genes in the "HLA Cluster" (Fig. 3). Helper (CD4) T cells are the T cells which link up to the HLA Class II molecules. Thus the "knowledge" phase that occurs from the presentation of HLA Class II requires the helper-function of setting up a general immune reaction involving cytokines, cellular and humoral protection against invasion by bacteria (or others). these function of HLA Class II in inducing a general immune system response is the cause how only "immunologically active" cells have to be active (B lymphocytes, macrophages, etc.) and not on all tissues while activating a general immune response (Devitt McH.O., 1985).

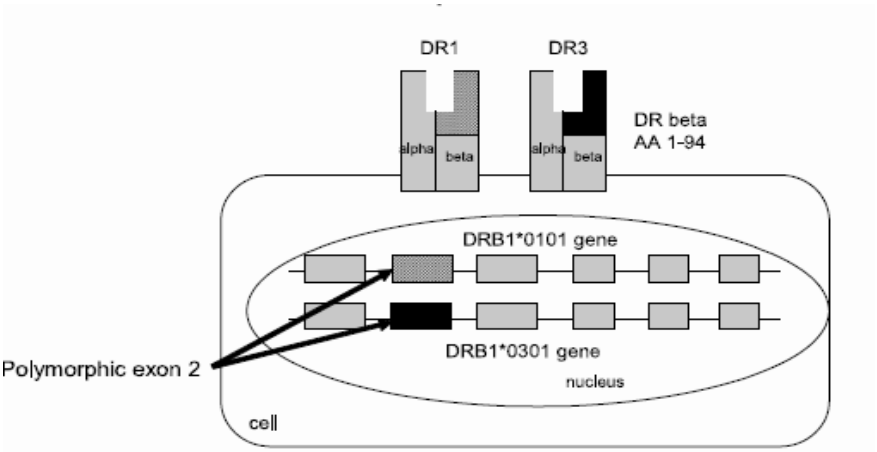


Fig. 3: DNA sequence difference leads to different HLA types expressed on cell membrane

GENETICS OF HLA

The alleles of Tissue typing distinguishes at **3** loci of HLA Class I (HLA-A, -B, and c) and the three loci of Class II (HLA-DR, -DP, and -DQ). Thus, when each chromosome is located twice (diploid) in each organism, 12 HLA antigens would be included in a typical tissue type of an individual (Paterlini P et al; (1991). These 12 antigens are co-dominantly inherited, Modern typing processes identify all 12 antigens, and for some, the presence of one does not impair typing. The genetic features of HLA antigens are various.

1. With polymorphism
2. HLA Inheritance
3. Disequilibrium Linkage
4. Reactivity to Cross

The HLA gene sequence varies significantly due to these genetic features and thereby contributes to the modification of the HLA antigen expressed on the surfaces of the cells (Fig.3)

POLYMORPHISM

The polymorphism is severe in the recognised HLA alleles. where it is the function of HLA molecules to introduce intrusive entity peptides, this severe polymorphism is likely to have developed as a tool to cope with all the different peptides that could be met with. That also is, in its amino acid sequence, each HLA molecule varies significantly from each other - this is what we're seeing as various HLA antigens. In the peptide binding cleft, the difference creates a slightly different 3-dimensional structure (U. Shankarkumar. 2004). It is important because the human population has a different varieties of HLA antigens to cope with all these peptides, that each have different formed peptide linking regions (clefts), so various peptides have different patterns and characteristics of charge . HLA antigens are also of considerable interest in anthropology studies. Populations that have frequencies of HLA antigen that are quite similar are specifically drawn from common shares (Imanishi et al. 1992; Clayton and Lonjou 1997).

NAMING OF HLA

Genes that present in the chromosome 6 at short arm of are strongly polymorphic, encoding HLA molecules. In this region, there are 200 genes with diverse functions. An entity responsible for naming these genes, allele sequences and their quality controls is the WHO nomenclature committee for the HLA factor system (Bhadran Bose. Et al, 2013). Here is an example of a nomenclature allele for its (Fig 4).

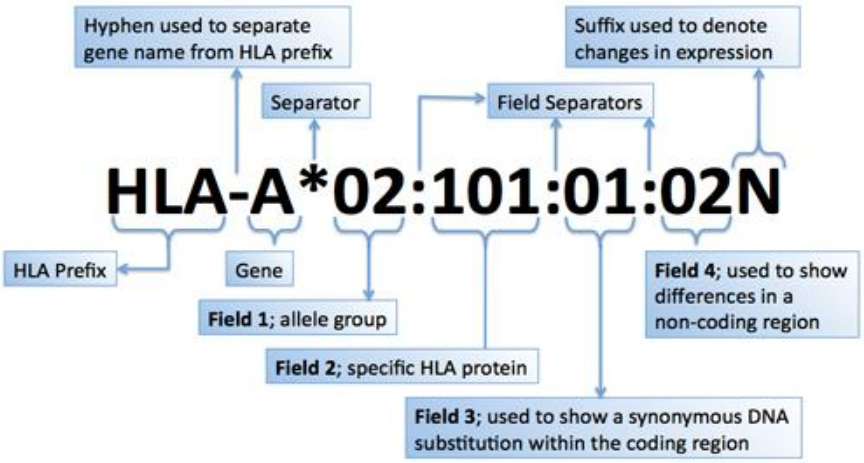


Fig. 4: Figure represents the nomenclature of HLA allele.

MOLECULAR PATHWAY OF HLA AND CANCER'S

Molecular pathway responsible for deficiency in HLA Class-I. In the proteosome, tumor antigens are processed and produce peptides that are transfer to the endoplasmic reticulum (ER) viaTAP; they bind to some heavy chain HLA Class-I in conjunction with beta2-microglobulin. Then the peptide HLA complex is transferred to the cell surface via the Golgi. Tumor "escape" from immune recognition is associated with many distinct defects. HLA Class-I gene transcriptional recognition defects result in HLA Class-I locus deficiency. Point mutation or identification of genes affecting the heavy chain HLA Class-I resulted in loss of HLA Class-I allele (Porta C et al., 1978).

POLYMERASE CHAIN REACTION(PCR)

Checking the PCR amplification function It shows its flexibility and its quality, too (Fig 5). Oligo-nucleotide primers are engineered to be complement with the ends of the amplifying chain and then combined with the DNA template in a molar surplus and deoxyribonucleotides in a sufficient buffer.

After heated, each of the oligonucleotides binds to a separate strand of the target segment to denature the initially strands and to cool to allow annealing of the primer. The primers are located such that the recombinant strands can overlap the binding site of the reversal oligo-nucleotide if one is elongated through the activation of a DNA polymerase. As the pathway of denaturation of DNA, annealing, and expansion of polymerase begins, the primers often bind in the newly synthesized strands to both of them initial DNA template and complementary sites and Fresh copies of DNA are extended. An explosive boost is the end product in the overall amount of genomic DNA composed of PCR primer sequences, which are mainly described at a potential amplification of 2n, while **n** represents the total of cycles.

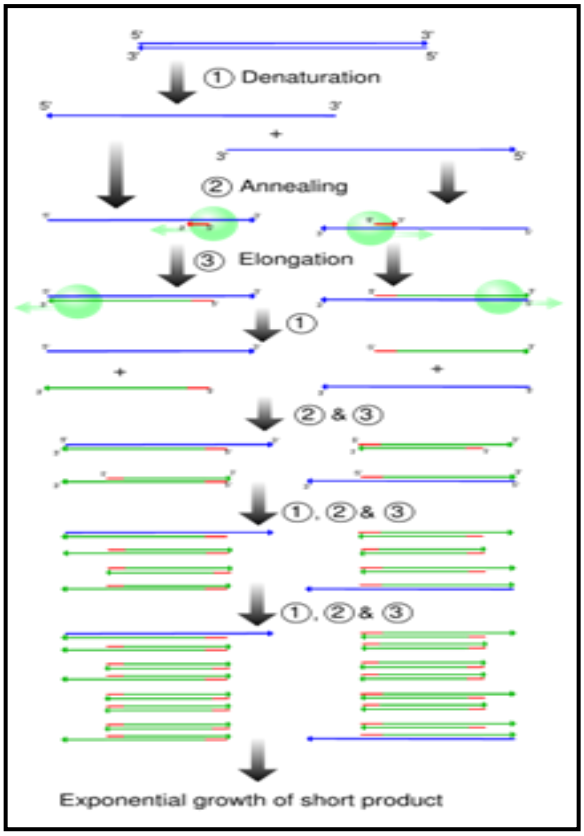


Fig. 5: Steps of PCR.

PCR Utility

The PCR system has two other features, addition to producing of double-stranded DNA shrapnel that can be produced by PCR, that contribute greatly to the benefit of PCR. First the binding location of the prefixes specifies the limits of the amplified portion and thus, PCR does not need the previous molecular cloning criterion for small nuclease recognition sites. PCR increases dramatically the versatility of selecting the size and orientation of the segment since there are a small number of DNA chain restriction sites. Second, the DNA template does not need to be completely complementary to PCR oligonucleotides. "Tails" can be attached to the end of the 5 "of the primer to connect sequences to the preparation positions that can be used to insert sites of recognition of endonuclease or other valuable sequences such as overgrown DNA mutations. This phenomenon enables PCR to appear as a form of rapid cloning of DNA.

To Occur PCR

In a certain orientation, the primers have to anneal (such that they point at each other). primers should have annealed Within acceptable range from each other (Fig. 6)

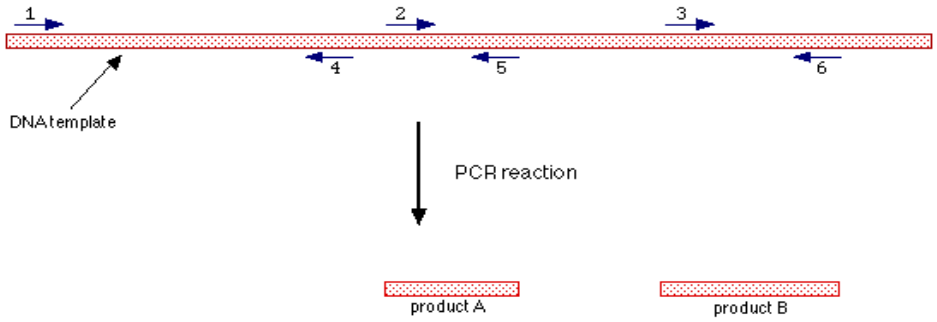


Fig. 6.

HLA PCR-SSP Method/ Sequence Specific Priming (SSP)

The widely used oligonucleotide primers induce PCR for this analysis, with sequences comparable to the target sequences that are typical of several specificities of HLA. For instance, the PCR for HLA-DR17 is unable to cause primers specific to HLA-DR15. The typing is completed with uses of variety of separate PCR's, each with primers specific to various HLA antigens.

Molecular Method - Sequence Specific Priming (SSP)

Primers oligonucleotide always had to begin the PCR. In this test the sequences are to be supplementary to recognized succession that is typical of several specificities of HLA. For e.g. primers unique to HLA-DR15 would not qualify for to incite the HLA-DR17 to PCR. The typing is completed with using a variety of different PCRs, all with primers unique for various HLA antigens.

2. MATERIAL METHODS

Requirements

Blood.

RBC lysis buffer

Ammonium chloride-9%,
Sodium bi corbonate-1%
EDTA-0.5M.

Nucleic Acid Lysis Buffer

Tris-Hcl-10mM
6M NaCl,
EDTA-2mM

Isolation of DNA from Blood

Take 4:4 ratio of Blood & RBC lysis buffer in to an eppendorf tube,
Then wet it for 3o minutes,
Then centrifuge at 3000 rpm for 10 minutes at 4^0c,
Collect the pellet, & add nucleic acid lysis buffer (800ul), then mix it,
Add 20ul of Proteinase K, & add 20% SDS (20ul) solution,
Incubate it over night at 37^0c,
Add 100ul of NaCl (6M) solution to the sample,
Then keep it -20^0c for 10 minutes,
Then centrifuge at 3000 rpm for 10 minutes at 4^0c,
Collect the supernatant slowly,
Add 200ul of isopropyl alcohol to the supernatant,
Then keep it -20^0c for night incubation,
Then centrifugation at 8000 rpm to 10 min at 40c,
Then wash the pellet with 70% ethanol,
Then centrifugation at 8000 rpm to 10 min,
Then discard the ethanol,
Add300ul of TE buffer to the pellet,
Then keep it at-20^0c for 1 hr,
Then go for gel running.

Preparation of Agarose Gel Electrophoresis

CHEMICALS

0.8% Agarose,
1XTAE,
Ethidium Bromide

PREPARATION

Weigh 0.8% of Agarose & mix in 20ml of 1x TAE buffer, heat the solution until agarose dissolved in buffer,
Allow the agarose cool to room temperature,
Add 3.5ul of Ethidium bromide in to agarose,
Mean while prepare the gel tank with help of tape,
Pour the agarose in to the gel tank & keep combs for well formed,
Allow the gel to solidify,
After solidification remove the combs slowly as well as the tape,
Keep the gel in 1xTAE buffer pre in the electrophoresis tank, check the volts &current volts should be 70v & current should be 15-40Amp,
Load the sample by adding BROMOPHENOL BLUE to the samples & load in to well's run the electrophoresis at 70v for 1hr,
Then observe the bands in UV illuminator.

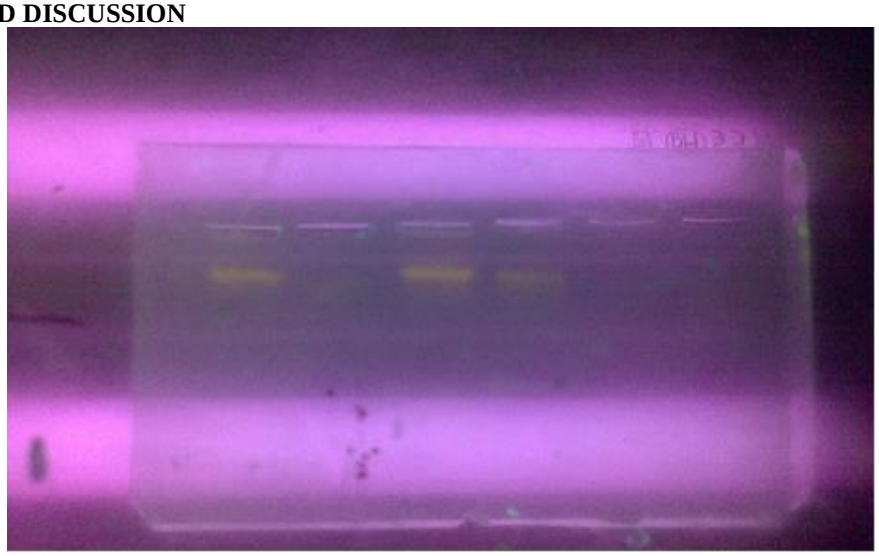
PCR_SSP program for HLA Allele for Normal and Disease Gene

Initial Denaturation Temperature	94^0 C	1min
Cyclic Denaturation Temperature	94^0 C	45 sec
Cyclic Annealing temperature	56^0 C	40 sec
Cyclic Extension Temperature	72^0 C	45 sec
Repetition for 32 cycles		
Final Extension	72^0 C	15 min
Hold	4^0 C	

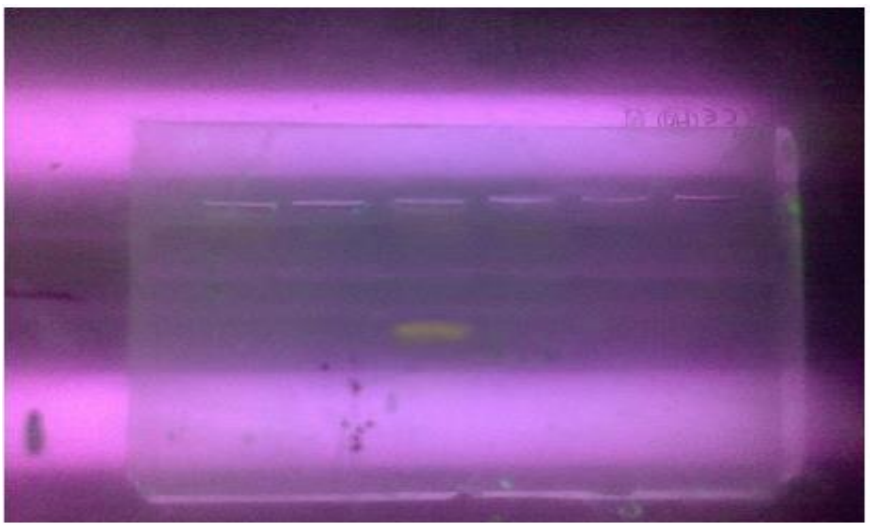
Master Mix Preparation

Total Volume	**25ul**
Water	14.7µl
15mM Taq Buffer	2 µl
25mM MgCl2	2 µl
dNTP	2 µl
100pm Primer1	1.2 µl
100pm Primer 2	1.2 µl
DNA	2 µl
5U/ul Taq Polymearse DNA	0.4 µl

1. RESULT AND DISCUSSION



Bands of Total Genome from Blood



Bands of HLA II Gene Amplification of Leukemia Blood Sample

From the results we found the expression of allele in patient is B0702 from the total genome of blood. This allele generates more chronicity and inflammation as comparable to the allele DRB301 expressed in Normal blood sample.

Thus we found the polymorphic allele which is the major cause for chronic inflammation found in a person during leukemia, the person has a chance to get cancer i.e. this person is susceptible to getting infection soon, can persist the infection for a long time and finally will get leukemia by various viral infection.

Another case can explain the presence of allele if recessive then the effect and susceptibility for that disease may not for the particular person but may affect his progeny.

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