Epigenomics in *Trypanosoma cruzi*: Exploring immunodetection techniques to study DNA methylation

Abstract
Trypanosoma Cruzi is probably on of the deadlies deseases in South America. A lot of studies have been done to understand the characteristics and behavior of this parasite. However, the unique characteristics of this dinoflagellate makes it challenging to prepare vaccines against it. In this study, we attempt to anwer questions about the gene expression profile of this parasite and futher study its methylation patterns. We will grow the parasite in the presence of a potent DNA methylation inhibitor (5-Azac). The gene expression profile will then be examined by microarray. An immunodetection technique will be used to detect methylation.

Introduction

The Chagas disease is still one of the most deadly diseases in South America today. The parasite responsible for this disease is the *Trypanosoma Cruzi* which is carried by the triatomine bug. The disease affects millions of people in central and South America [1]. One intriguing characteristic of the parasite is its ability to thrive and replicate within muscle cells for 30 years before killing its host. In 30% of the cases, individuals develop severe damage to the digestive tract, cardiac and smooth muscles. The disease agent exhibits three phenotypic characteristics during its life cycle. These include the amastigote form, which is found inside of the host cell and is replicative, the epimastigotes which are extra cellular and also replicative and then the infective form of the protozoan, the trypomatigote [2]. A lot of research efforts are being made to understand the complex biological functions of this parasite. However, information on the epigenomic events like DNA methylation is limited.

While some data show that methylation in trypanosomes have some kind of effect on DNA protein interaction and DNA conformation and subsequent effect of gene expression, the extent of the effect is still not known; specific genes that are up regulated or down regulated in the presence of a demethylation agent like (5azaC) and the extent to which it affect the parasitic ability of the protozoan remains unknown.

It has been found that there are methylated bases in the *T.Cruzi* genome specifically, 5-methylcytosine (m_5Cyt) and possibly N6- methyladenine (m_6Ade) [2]. The occurrence of methylated bases are in sites that are different from CpG sites as they are characterized in other organisms. In *T. Cruzi*, methylated bases are found in regions possibly XCGX. [2]. Building on some of this evidence, our quest this past summer was to identify genes that are under the control of a Methylation. In this task, we sought to block methylation with the use of a potent demethylation agent, namely 5-azaCytidine (5-azaC). The drug works by blocking the action a DNA methyltransferase that works tranfering a methyl group to the DNA. The DNA methyltransferase use S-ademosyl methionine as a methyl donor.

Methods

Several experiments that have been done with Trypanosomes have used different methods to detect the stutus of methylation in this this parasite. Interestingly, different methods have yeilded different results. In our previous experiments we used microarray technology to detech upreglation and downregulation of certain genes, the downside of this technique is that specific genes and the location of the genes that were under the control of methylation was not known. In this experiment we would use an immunodetection technique that would use a specific antibody prepared against methylated cytosine in the DNA of the parasite. This techniques has been used to detect methylated cytosines in the genomic DNA of *Drosohila melanogaster*[x1]. In order to confirm the methylated status of the identified genes

they would treated with sodium bisulfite.

Previous Results

Using the methods describe above, we found that there were about 1035 genes under the contol of the demethylation inducing drug 5-Azac. These genes were upregulated, upon further analyses, we realized that most of these genes were cycline associated proteins. This came as no surprise because it corrolates with our earlier observation during the parasite growth proccess. We observed that after addition of the drug, therer was a proliferation of the parasites. This has been observed in other studies also [2].

Discussion.

Most studies that have been conducted in parasitic methylation have yeilded different results. While some have noted the incidence of methylation in T. Cruzi, others have note found the same results working with the same parasites. The only method that has not been significantly tested in trypanosomes in an immunodetection technique which can definitely detect the incidence of methylation in this parasite. An anti-body 5mc is mostly used to detected methylated cytosines.The cycline associated genes that were observed are mostly associated with parasite growth.

References:

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