**Name:** Ayesha Anwer **Notification No:** 522/2022

**Name of Supervisor:** Dr. Syed Naqui Kazim **Date of Award:** 26-09-2022

**Department/Centre:** Centre for Interdisciplinary Research in Basic Sciences (CIRBSc)

**Topic**: Viral Promoter Mediated Expression of siRNA and Inhibition of Polymerase Gene of Hepatitis B Virus

## Finding

Hepatitis B virus is a global concern that can cause acute or chronic infection of the liver, at present approximately 3.5% of the world's population is chronically infected. Presently available treatments have failed to give a satisfactory result. This failure in successful therapeutic outcome is mainly due to emergence of anti-viral resistant mutations on prolonged usage of NAs. Adverse side effects are also the reasons for the unsatisfactory outcomes of the available therapeutic regimens. siRNAs discovery appears to be a possible ray of hope in the search for better treatment options when the present-day anti-viral therapies are partially effective. Even though siRNA has the remarkable ability to silence disease causing specific genes, it faces several challenges. Successful application of RNAi mainly depends on effective and specific targeting sequence and a good vector expressing the corresponding siRNA or shRNA. Like generalized mechanisms involved in gene expression, siRNA also works under the tight control of regulatory elements more particularly the role of promoter which also appears to be relatively more important. Enhanced siRNA expression can be achieved by using a strong promoter. Viral regulatory elements play an essential role in the pathogenesis of the disease. Use of liver specific promoter will restrict the expression to hepatocytes. This will also provide information about the crucial role a promoter could play for an enhanced siRNA expression. This might lead to an enhanced reduction in the copy number of viral genome targeted by siRNA. Hence, it is hypothesised that liver specific

regulatory elements either of the host or virus may play a pivotal role in specific inhibition and gene expression process of HBV. Adequate siRNA expression to inhibit the HBV genome can be tuned by choosing the right promoter. Moreover, we expect that by carefully choosing the combination of sequences of the target genes of HBV, higher therapeutic efficacy can be achieved with the help of proposed approaches in this research proposal. Thus, by coupling the regulatory elements-based strategies with the appropriate design of siRNA constructs harbouring single, double or multiple targets of HBV genome sequences would be an important milestone in the field of hepatitis B virus novel therapeutic approaches. **Results**: pEGFP and pSilencerCMV-GFP vectors resulted in highest expression of the GFP gene, but its expression was non-specific, irrespective of the origin of the cell lines. This indicates that CMV promoter is a ubiquitously active strong promoter. pSilencerHBV-GFP construct resulted in a stronger expression of GFP in Hep3B cell line than pSilencerU6-GFP indicating the tissue specificity of the HBV core promoter. Thus it can be concluded, novel chimeric vector harboruing HBV core promoter demostrated nearly seven folds increase in expression of the reporter gene in cell line of hepatic origin suggesting the significance of promoter in enhancemnt of tissue specific expression. siRNA expression driven by HBV core promoter inhibited the expression of reporter GFP gene (2.4 folds) in Hep3B cells (liver cell line) suggesting the significance of promoter in enhancement of tissue specific inhibition. Selection of Y63 motif as the target sequence within HBV genome in order to inhibit the viral life cycle coupled with exploitation of liver specific promoter (siRNAHBV-PolY63) could be the most appropriate strategy for siRNA mediated inhibition of HBV. Targeting of the primase domain of polymerase demonstrated highest degree of HBV inhibition with respect to secretory antigens (HBsAg and HBeAg) and viral DNA.

# Scholar Profile

## Personal Information:

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| Name: | Ayesha Anwer |
| Father Name: | Dr. Mohammad Anwer Alam |
| Date of Birth: | December 05, 1990 |
| Sex: | Female |
| Address: | Lab-125, Hepatitis B virus laboratory, Ramanujan block, Mujeeb Bagh, Jamia Millia, New Delhi, India |
| Nationality: | Indian |
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## Academic Qualification:

- Master of Science in Biochemistry (2014) with 1st division from Hamdard University,Delhi, India.
- Bachelor of Science in Bioscience (2012) with 1st division from Jamia Millia Islamia, Delhi, India.
- Higher Secondary Examination (2009) with 1st division from Jamia Millia Islamia, Delhi, India.
- Secondary Examination (2007) with 1st division from Cambridge International Examinations, British Council, Jeddah, Kingdom of Saudia Arabia.

## Fellowships and awards

- ICMR- SRF: 10th July 2019 to 9th July 2022.

## Declaration:

I hereby declare that all the statements made in this application are true and complete to the best of my knowledge.

Date: (Ayesha Anwer)