HSV in Cervical Cancer

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Introduction

There are an estimated 11,000 new cases of cervical cancer (CaCx) diagnosed in America each year (American Cancer Society) making it one of the most commonly diagnosed cancers in women. Currently, there is compelling evidence that some strains of HPV are intimately involved in the development and maintenance of CaCx. Research has also indicated that HSV2 may act as a potential cofactor. However the evidence for HSV being a cofactor is not as compelling as HPV. Unlike HPV, HSV proteins and DNA cannot be readily and consistently detected in tumor samples and no mechanism for how HSV sequences could be involved in CaCx has been demonstrated. For these reasons, research on HSV as a causative agent in CaCx has waned substantially.
The current state of molecular technology now allows us to reexamine the role of HSV, particularly in light of the role that small RNAs known as microRNAs may play in the development of CaCx (Wang et al., 2008). My laboratory has now identified an 83bp region of HSV2 that can transform cells when it is under the control of an eukaryotic promoter and has demonstrated that this region is present in a high proportion of CaCx cell lines

Methods

Polymerase chain reaction: PCR was performed using 3 different thermo stable DNA polymerases, Taq, Q5 and Dynazyme, according to manufacturers specifications (New England Biolabs and Thermo Scientific). Conditions were varied to establish the optimal times and temperatures for amplification.
Primers: Primer design was accomplished by using the Primer 3 program. Primers were synthesized by Life Technologies
Cell lines and tumor samples: Cell lines were obtained through ATCC and cultured in DMEM supplemented with 10% fetal calf serum. DNA insolation: Genomic DNA was isolated using a kit obtained from Life Technologies according to their specifications. DNA was quantitated by spectrophotometry at 260nm.

Results

Sau 3A1 fragments of HSV2 G were cloned into the pCDNA3.1 myc/his. Lipofection of this library into NIH3T3 cells resulted in several foci. Sequencing of the HSV2 DNA from these foci revealed that the HSV 2 G DNA in these cells was identical to a previously identified region of HSV2 that could transform DNA. To further confirm the ability of this region to transform cells oligonucleotides that encompassed this region were synthesized and cloned into the vector pSuperior neo. Retransfection of this clone into NIH3T3 cells also resulted in foci (Fig 1)

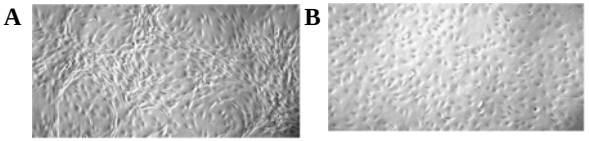


Figure 1. Foci formed by transfection of control vector with synthetic oligonucleotides of HSVmir9 (A) or an empty control vector (B).

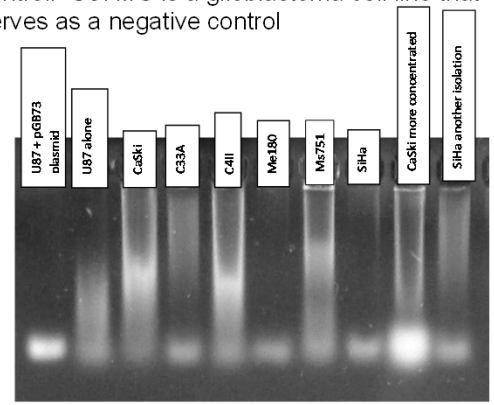
To determine if these sequences were present in CaCx cell lines we synthesized a series of forward and reverse primers for use in PCR. Design of these primers was through the use of Primer3, a web based primer prediction program available through the Massachusetts institute of Technology. (Table 1)

Forward Primers	Sequence
91	CCGAGCGGTACTTCTACACC
142	TCCCTCAGCATCCTGAACCG
25	CGCGGATCCAGCACCGACCCCCTAGATAC
5.3	GAGCTCGTGTTCGTGG
Reverse Primers	
257	AAGGCAAACAGAAAGCGGTAG
680	CGCTCTAGATAGCACGAGGCTGTCGTATG
704	CGCTCTAGACCCCCGAGGTAGTTGTTGTA
3.2	GGTAGAAGCCGAGCTCGC
3.3	CTCGCCCTCGGAGAGC
H3	GTTTTCCGTCACCAGGTCGT

Table 1. Sequences of primers

Results

Fig 2. Agarose gel electrophoresis of PCR products from different CaCx cell lines. Primers were used for amplification of target sequences from different CaCx cell l.ines. pGB73 is a plasmid that contains the target sequences and was used as a positive control. U87MG is a glioblastoma cell line that serves as a negative control



Conclusions and future direction

PCR can be used to detect HSV sequences ~50% of the CaCX cell lines surveyed - a much higher percentage than what has been detected before.

This sequence contains a hairpin loop like structure that is similar to miRNAs

Short RNA reads from other laboratories contain HSV identical sequence from this region as well

We will need to demonstrate the targets for this putative novel miRNA

Aknowledgements

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