Introduction

Liver cancer is a leading cause of cancer deaths worldwide, accounting for more than 600,000 deaths each year The American Cancer Society's estimates -of primary liver cancer and intrahepatic bile duct cancer in the United States for 2016 are about 39,230 of newly diagnosed cases - and 27,170 died people of these cancers[i] .In Egypt; Liver cancer is a serious if not the most serious cancer problem. It is ranked the first among cancers in males (33.6%) and next to breast cancer among females based upon results of National Cancer Registry Program (NCRP 2008-2011)[ii]. The rising rates of HCC in Egypt are due to the high prevalence of hepatitis B virus (HBV) and hepatitis C virus infection (HCV) among Egyptian population[iii]. Therefore; we need effective strategies for early detection and better management of HCC which will be of great value in developing countries with limited resources and high incidence rates of HCC, such as Egypt.

It is well known that the human genome is actively transcribed; however, there are only about 20, 000 protein-coding genes, accounting for about 2% of the genome, and the rest of the transcripts are non-coding RNAs including microRNAs and long non-coding RNAs (lncRNAs). LncRNAs play an important part in the regulation of gene expression, including chromatin modification, transcription and post-transcriptional processing. It has been confirmed that dysregulation of lncRNAs is accompanied by a number of human pathological diseases, mainly tumors.[iv]

A most commonly used approach for gene functional study is knockdown by RNA inference (RNAi). However, many lncRNAs are localized to the nucleus ([v]), which can make it difficult to achieve robust knockdown. Thus, genetic editing at the genomic level provides a better alternative because it targets the genomic DNA. There are several genetic tools available for this purpose, including zinc finger nuclease (ZFN) and transcription activation-like element nuclease (TALEN) ([vi]). Recently, a novel genetic engineering tool called clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system is more advanced because of

easy generation and high efficiency of gene targeting. Importantly, it only requires changing the sequence of the guide RNA (gRNA).

CRISPR/Cas9 has rapidly gained popularity due to its superior simplicity ([vii]). In this system, a single guide RNA (sgRNA) complexes with Cas9 nuclease, which can recognize a variable 20-nucleotide target sequence adjacent to a 5′-NGG-3′ protospacer adjacent motif (PAM) and introduce a DSB in the target DNA ([viii]). The induced DSB (DNA double stranded break) then triggers DNA repair process mainly via two distinct mechanisms, namely, the non-homologous end joining (NHEJ) and the homology-directed repair (HDR) pathways.

CRISPR loci and their associated *cas* (CRISPR-associated) genes provide adaptive immunity against viruses (phages) and other mobile genetic elements in bacteria and archaea. While most of the early work has largely been dominated by examples of CRISPR-Cas systems directing the cleavage of phage or plasmid DNA, recent studies have revealed a more complex landscape where CRISPR-Cas loci might be involved in gene regulation.

In human cells, efficient knock-in of foreign DNA into a selected genomic locus is anticipated to facilitate various applications, ranging from gene

function study to therapeutic genome editing. Currently, most studies have focused on HDR-based strategies ([ix],[x]).In a recent study by Merkle *et al*., the efficiency of CRISPR/Cas9-induced HDR-mediated knock-in was estimated to be around 1×10^{-5} without pre-selection ([xi],[xii]).

Scientists constructed a universal reporter system, by targeting the *GAPDH* locus in human genome with a promoterless fluorescent reporter. Through systematic investigation into the potentials of both HDR and NHEJ repair in mediating CRISPR/Cas9-induced reporter integration, researchers demonstrated that CRISPR/Cas9-induced NHEJ can mediate reporter knock-in more efficiently than HDR-based strategy, in various

human cells types including human ESCs. This finding paves a new path for efficient genome editing in human ESCs and somatic cells, and it offers a great potential in their subsequent applications.([xiii])

Aim of the work

1- To analyze circRNA and disease databases to select significantly relevant circRNA for HCC.
2- To analyze circRNA- miRNA interaction databases to retrieve competing endogenous RNA specific for HCC
3- To characterize the expression of the serum cirRNA-associated ceRNA genes in HepG2 cell line to evaluate their role in pathogenesis of HCC.
4- To compare beteen the efficacy of a CRISPR-Cas9 & non Cas 9 based synthetic circuit on modulating cirRNA-associated ceRNA related HCC expression using HepG2 cell line.

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