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Investigating the Role of MicroRNAs in The Pathogenesis and Progression of Hepatocellular Carcinoma

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Abstract

The primary regulators of the cellular pathways implicated in virus-induced pathogenesis include a variety of host factors, including proteins and microRNAs (miRNAs). Numerous secretory chemicals that are secreted from virus-infected cells aid in cell-to-cell communication and foster a microenvironment that promotes cellular pathogenesis, both of which advance the course of the disease. One of the main parts of secretive substances is exosomes. They are the small extracellular vesicles, secreted out by the cells and have a significant role in cell-to-cell signaling. They typically represent the state of the cell and are made up of several host components such as cellular proteins, lncRNAs, mRNAs, and miRNA. Exosomes, which are vesicles that originate inside cells as multivesicular bodies and are ejected from the cell upon fusion with the cell membrane, will exhibit any changes in the composition of the cell. All things considered, the work shows that exosome proteins and miRNA may play a part in host response and viral tactics, which may together accelerate the course of the disease and provide new targets for potential treatment.

Keywords: MicroRNA, Hepatocellular Carcinoma, Pathogenesis, Biomarker, Therapeutic Target.

1 INTRODUCTION

Although there is no information available regarding JIA-ERA patients, dysregulation of miRNAs has been shown in a number of inflammatory disorders, and polymorphisms in miRNAs and their targets are also linked to inflammatory disorders, including arthritis. Additionally, the overexpression of TLRs in JIA-ERA may indicate that miRNAs implicated in the TLR pathway play a part in JIA-ERA. The main risk factors for HCC are hemochromatosis, autoimmune hepatitis, alcoholic cirrhosis, non-alcoholic fatty liver disease, aflatoxin B1, and chronic HBV and HCV infection (Callegari et al., 2013). Liver transplantation, percutaneous ablation, radioembolization, transarterial chemoembolization (TACE), systemic chemotherapy, and surgical resection are among the current curative and palliative therapeutic approaches for HCC. The only traditional molecularly targeted treatment for advanced HCC is the tyrosine kinase inhibitor sorafenib. Several chemotherapy medications are being used in monotherapy or polytherapy regimens, including doxorubicin, cisplatin, and 5-fluorouracil. Systemic therapies for HCC include immunotherapy, hormone therapy, and chemotherapy (Gramantieri et al., 2008). The prognosis for HCC is still dismal even with the introduction of numerous therapy approaches, including clinical treatments and antiviral medicines. Therefore, the

development of new diagnostic and therapeutic alternatives is necessary. Over the past ten years, scientists have concentrated on examining the molecular (Morishita et al., 2021) foundations, including medication target locations, biomarkers, and chromosomal abnormalities. Among the molecular entities, miRNAs have garnered significant attention in recent years due to their significant therapeutic (Callegari et al., 2015) potential and pivotal role in carcinogenesis.

Hepaciviral, Flavivirus, Pest virus, and Pegi viruses are the four genera that make up the family Flaviviridae. Hepatitis is linked to the genus Hepaciviral. This genus includes the blood-borne disease Hepatitis C virus (HCV), which infects humans and causes chronic illnesses. On the other hand, flaviviruses cause acute infections and are spread by arthropods, usually mosquitoes. They have developed the ability to multiply in the cells of both insects and animals. Significant human infections that are members of the Flavivirus family include the Dengue, Zika, and yellow fever viruses. The third genus of the Flaviviridae family, which includes viruses with veterinary applications, is called pest virus. Usually, it affects cattle and animals. Three notable examples of pest viruses are the classical swine fever virus (CSFV), the bovine viral diarrhea virus (BVDV), and the border disease virus (BDV). Very little is known about the role of Pegi viruses in disease and their natural animal host, despite the fact that they produce persistent infections.

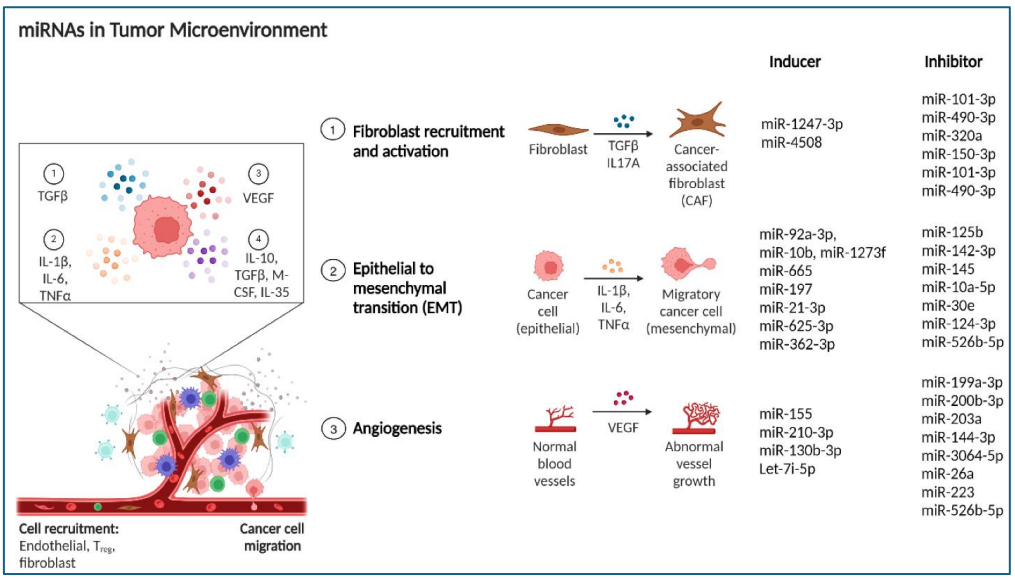

Figure 1: Role of MiRNAs in the Pathogenesis

Numerous miRNAs have been discovered to be dysregulated in HCC, and certain miRNAs have been linked to clinicopathological characteristics such as metastasis, recurrence, and prognosis. Furthermore, strong evidence indicated that miRNAs target tumor cell growth, death, and metastasis in order to affect the development of HCC. Figure 1 showed the suggested mechanisms by which miRNAs function as tumor suppressor genes or oncogenes. A schematic representation of the deregulated miRNAs, their target genes, and the ensuing phenotypes of the resulting HCCs is provided by the up-regulated and down-regulated miRNAs in HCC. This study's objective is to systematically assess the

differentially expressed miRNAs in HCC patients that have been reliably identified by another research. We also go over the clinical uses of miRNAs in the diagnosis and management of HCC.

2 HEPATOCELLULAR CARCINOMA

Cancer may develop as a result of aberrant cell division and expansion brought on by the buildup of mutations. The rapidly proliferating cancer cells that are dividing uncontrollably create the lumps or tissue mass in a solid tumor. The tumor turns malignant when cells spread to other tissues, but the benign solid tumor remains contained in the original tissue. Tumor suppressors and proto-oncogenes lose their ability to regulate cell growth and division as a result of gain-of-function and loss-of-function mutations, which can result in cancer. Liver cancer can manifest as hepatoblastoma, intrahepatic cholangiocarcinoma, or hepatocellular carcinoma (HCC). The type of liver cancer that occurs most frequently is hepatocellular carcinoma. Alcoholic cirrhosis, non-alcoholic fatty liver disease, and persistent HBV and HCV infection are the primary risk factors for HCC. According to Singh et al. (2014), chronic HBV and HCV infections are linked to an elevated risk of HCC that is 5-100 times and 15-20 times higher, respectively. One in four instances in the US are caused by HCV infection, which carries the highest relative risk. Approximately 71% of liver cancer cases, however, are thought to be possibly avoidable. This is due to the fact that the primary risk factors—such as hepatitis B and C viruses, obesity, smoking cigarettes, and excessive alcohol use—can be changed. A frequent gastrointestinal tumor, HCC has a poor prognosis and a high degree of aggressiveness. HCC development is a multi-step, intricate procedure. Chronic liver disease (CLD) is characterized by chronic inflammation and hepatocyte regeneration, which create an environment that is conducive to the accumulation of genetic and epigenetic alterations, microenvironmental changes, and stem/progenitor cell proliferation, all of which may lead to the development of liver cancer. Generally speaking, persistent liver inflammation brought on by risk factors can lead to both acute and chronic necroinflammation, which can then develop into fibrosis. Extracellular matrix is deposited in the liver in cirrhosis, resulting in the formation of scarred tissue. The liver develops malignant tumors at the stage of hepatocellular carcinoma (Khan et al., 2022).

3 ROLE OF MICRORNAS IN CHEMORESISTANCE

One method to determine the function of miRNA in drug resistance is to compare the expression profiles of human tissue or HCC cell lines with those of their drug-resistant subline. A study treated the Huh7 cell line with doxorubicin, cisplatin, carboplatin, mitomycin C, and vincristine at escalating concentrations in order to create drug-resistant sublines from HCC cells and examine the expression profile of microRNAs in the treated cells. Drug-resistant sublines have distinct microRNA (Vasuri et al., 2018) expression profiles, according to microarray research. Results indicated that 53, 56, 58, 58, and 49 miRNAs were upregulated and 52, 50, 41, 55, and 56 miRNAs were downregulated in Huh7 cells treated with doxorubicin, carboplatin, cisplatin, mitomycin C, and vincristine. Of these, doxorubicin-, carboplatin-, cisplatin-, and mitomycin C-induced simultaneous upregulation of 26 microRNAs and

simultaneous downregulation of 25 microRNAs were observed. On the other hand, the vincristine-treated Huh7 and the other sublines shared just 12 upregulated and 13 downregulated microRNAs. This demonstrates that several microRNAs are triggered by the vincristine mode of action. However, doxorubicin and docetaxel were used to treat the MCF7 cell line in a related investigation on the breast cancer cell line. Microarray analysis was used to examine the expression profiles of the cell line and its two resistant variations. The findings demonstrated that, in contrast to the parent cell line, 183 microRNAs were expressed differently in the two resistant sublines (Oura et al., 2020; International, 2021). Subsequent research revealed that miR-222 and -29a may contribute to MCF7 acquired resistance to doxorubicin and docetaxel via regulating PTEN. A different study looked into the expression of microRNAs in a cell line that was resistant to paclitaxel. MiR-125b, miR-221, miR-222, and miR-923 were found to be increased in cancer cells resistant to paclitaxel. Additionally, miR125b was demonstrated to inhibit the expression of pro-apoptotic BCL-2 antagonist killer 1 (BAK1), which decreased paclitaxel-induced apoptosis and increased resistance as a result (Zhou et al. 2010). Numerous microRNAs have been shown to have a part in chemoresistance in HCC patients.

4 MATERIALS AND METHODS

Cell Lines

This study made use of two human hepatoblastoma cell lines (HepG2) and hepatoma cell lines (Huh7) that were supplied by the Institute of Liver and Biliary Sciences in New Delhi, India.

Cell Culture and Growth Conditions

Both cell lines were kept in a humidified incubator at 37˚C with 5% carbon dioxide in high-glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS: Gibco) and 1% penicillin/streptomycin (Gibco).

Determination of Drug Inhibitory Concentrations by MTT Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used to identify medication concentrations that resulted in cell growth inhibition of 20% (IC20), 30% (IC30), 40% (IC40), and 50% (IC50). 2 mg of MTT (Merck Millipore) was diluted in 1000 µl of PBS to create the MTT stock solution. As advised by the manufacturer, the tube was wrapped in foil and kept at -20 oC for a month. Prior to organizing the full-scale experiment, the MTT assay was used to determine the proper density of seeded cells using a serial dilution in triplicates for 48 hours. For both cell lines, the ideal cell density was 1×104 cells/well, which resulted in absorbance of approximately 0.9 to 1. After 48 hours, the MTT assay was used to determine the cell viability. Pre-incubation with 2 µM DAPT for 24 hours was determined to be the ideal dose and duration based on these two tests. Therefore, the cells were first pre-incubated with 2 µM DAPT for 24 hours before being treated with the IC50 dosages of doxorubicin, cisplatin, vincristine, or 5-fluorouracil for 48 hours in all experiments to block the NOTCH signaling pathway.

MiRNA Transfection

As previously mentioned, the Huh7 cells were sorted using FACS after being labeled with an anti-human APC-CD133 antibody. The freshly sorted CD133+ cells were transfected with an inhibitor or miRNA mimic using the reverse transfection technique (Okazaki et al. 2007). Using this technique, the cells and media were added after the complexes had been produced inside the wells. As directed by the manufacturer, lipofectamine 2000 (Invitrogen, cat. no. 11668-027) was used to transfect the miRCURY LNA miRNA inhibitor ACCTATCCTGAATTACTTGA (Exiqon) and miRCURY LNA miRNA mimic TTCAAGTAATTCAGGATAGGT (Exiqon). The imitation miRNA-Lipofectamine complex was prepared by plating 15 pmol of mimic miRNA in a 6-well plate after diluting it in 100 µl of Opti-MEM (Gibco). The wells were then filled with 3 µl of lipofectamine. 150 pmol of inhibitor miRNA was diluted in 100 µl of Opti-MEM to create the inhibitor miRNA-lipofectamine complex. Then, 5 µl lipofectamine was added to the wells.

Statistical Analysis

GraphPad Prism software (Graph Pad Software Inc., USA) was used to analyze the data using a two-tailed unpaired student t-test and a one-way ANOVA. At $P<0.05$, differences were deemed statistically significant. The mean ± standard deviation was used to present the results. (SD).

5 RESULTS

The mRNA level of SOX2 was upregulated in cells treated with DOX (24-fold), CIS (19.3-fold), VIN (8-fold), and 5-FU (10.1-fold); however, all DAPT+DOX (0.06-fold), DAPT+CIS (0.23-fold), DAPT+VIN (0.81-fold), and DAPT+5-FU (0.023-fold) cells had significantly lower levels after pre-treatment with DAPT ($p<0.05$) (Figure 2).

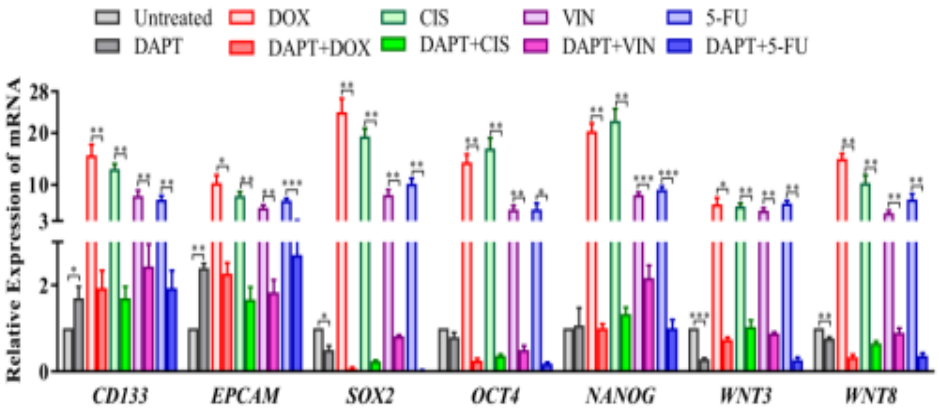

Figure 2: The Expression of Stemness-related Genes in Drug-treated Cells

OCT4 gene expression was elevated 14.3-, 17-, 5.1-, and 5.2-fold in DOX, CIS, VIN, and 5-FU-treated cells, and dramatically decreased to 0.25-, 0.36-, 0.5-, and 0.18-fold in DAPT+DOX, DAPT+CIS, DAPT+VIN, and DAPT+5-FU cells ($p<0.05$) (Figure 2). In cells treated with DOX (20.3-

fold), CIS (22.3-fold), VIN (8-fold), and 5-FU (9-fold), the mRNA level of NANOG was upregulated; however, in all DAPT+DOX (1-fold), DAPT+CIS (1.3-fold), DAPT+VIN (2.1-fold), and DAPT+5-FU (1-fold) cells, pre-treatment with DAPT significantly decreased it ($p<0.05$) (Figure 2). Cells treated with DOX, CIS, VIN, and 5-FU showed 6.2-, 5.8-, 5-, and 6.4-fold increases in WNT3 gene expression, but cells treated with DAPT+DOX, DAPT+CIS, DAPT+VIN, and DAPT+5-FU showed a dramatic decrease to 0.74-, 1-, 0.88-, and 0.26-fold increases.

6 CONCLUSION

Seventy to ninety percent of instances of primary liver cancer are hepatocellular carcinoma (HCC). Chemotherapy is a palliative measure to increase the survival rate of patients with HCC in addition to liver transplantation and surgical resection. For advanced HCC, sorafenib is the only conventional molecular-targeted treatment available among chemotherapeutics. However, sorafenib and other chemotherapeutics, such as cisplatin (CIS), doxorubicin (DOX), vincristine (VIN), and 5-fluorouracil (5-FU) medications, do not work well for HCC when used alone or in combination. As a result, the recurrence phenomena persists and chemotherapy-based treatment for HCC is not curative. The chemoresistance of cancer stem cells (CSCs), especially those that express the surface glycoprotein CD133, which has been shown to have tumorigenicity, the potential to start and maintain tumors, and a high capacity for self-renewal, is partly responsible for this.

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