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The Utility of Circulating microRNAs as Biomarkers in Viral Infection-Associated Cancers

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Abstract

Since their discovery, the role of microRNAs (miRNAs) has been expanding. These small, non-coding RNAs have been associated with gene regulation by binding to 3' UTRs. Specific miRNA expression profiles are associated with cancer development and may serve as markers of malignancy. Many of these cancers are also associated with viral infections. As some viruses express their own miRNAs, research has begun to investigate if cellular or viral-encoded miRNAs could also serve as specific markers of these virus-associated cancers. Discovery of miRNAs in both tissues and serum have been reported in patients with virus positive tumors. These miRNAs may emerge as important, non-invasive diagnostic tools for detecting tumor development and progression and improving cancer treatment.

Keywords virus, cancer, miRNA, biomarker, serum

Introduction

It is estimated that approximately 20% of cancers worldwide are due to infectious agents (1). These pathogens contribute to multiple cancer types. The most common viruses associated with cancers include Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell leukemia virus-1 (HTLV), human papillomaviruses (HPV), Kaposi's sarcoma herpes virus (KSHV), and human immunodeficiency virus, type 1 (HIV-1; 2-3). The problem with determining a causal relationship between many of these cancers and viral infection is that the tumors often appear years after the initial infection or the virus alone is not the causative agent of the cancer (4). Many of these infectious agents are prevalent at high percentages in the general population and thus require co-factors to contribute to cancer development. This makes use of the virus itself as a diagnostic tool for cancer development limited. Initial studies on virus-specific markers in cancers have focused on the presence of viral transcripts and/ or antibodies, or changes in gene expression seen only in virally infected cells. The use of other, more specific biomarkers in response to viral infection may improve understanding of the relationship between these viruses and their associated cancers.

MicroRNAs (miRNAs) are small 19-24 nucleotide non-coding RNAs that bind to the 3' UTR of complementary transcripts and are important in transcriptional regulation. Analysis of miRNA expression levels has been described for use in cancer treatment, diagnosis, and characterization (5). These RNAs play an important role in regulating cellular gene expression. In cancers, these miRNAs often target oncogenes, immune regulatory genes, cell motility, or other important sequences for cellular transformation and survival. Alterations in miRNA levels have been reported in a multitude of viral infections (6). Furthermore, some viruses also encode their own miRNAs (7). Many studies have identified miRNA expression patterns that

are specific to viral infection or cancer development.

The possibility of using miRNAs as biomarkers was further expanded by the discovery of miRNAs in serum and plasma samples (8). Many of the cancers evaluated for tumor-specific miRNA expression patterns are also associated with virus infections. Detection of these virus-associated miRNAs may give information on pathways that can be targeted in cancer treatment, or act as a specific biomarker of cancer cells. Table 1 summarizes the viruses reviewed in this report, the cancers they are associated with, and miRNAs identified as potential serum biomarkers. This paper will further summarize studies investigating specific miRNAs as minimally invasive, specific biomarkers in the serum of patients with virus-related cancers.

Epstein-Barr virus, lymphoma, and nasopharyngeal carcinomas

Also referred to as human herpesvirus 4 (HHV-4), EBV is a common human DNA virus associated with multiple cancers including Hodgkin's, non-Hodgkin's, and Burkitt's lymphoma as well as nasopharyngeal carcinoma (9). Many of these cancers occur in the conjunction with HIV co-infection. Infection is passed through the saliva, and the virus persists in latency for the remainder of a person's life. The role of EBV in these cancers is varied depending on cancer type, and tumor development is associated with expression of a multitude of viral transcripts, immune suppression, and/or co-infection with another virus (9). Because a large percentage of the world's population is infected with EBV, establishment of a biomarker distinguishing individuals who may develop EBV-specific cancers would improve early detection of these malignancies.

Using miRNA expression levels in normal versus cancerous cells, multiple viral and host miRNAs have been associated with EBV-infected tumors (10). To date, few studies have investigated the presence or correlation of these miRNAs in serum from patients with EBV-associated cancers. Wong et al., detected six EBV encoded

Table 1: Virus associated cancers and reported miRNA serum markers.

Virus	Associated Cancer	miRNA Serum Markers
EBV	Nasopharyngeal carcinoma	BART6-5p*, 6-3p*, 14*, 18-5p*, 19-3p*, 2-5p*
HBV	Hepatocellular carcinoma	2, 101, 122, 223, 375
HCV	Hepatocellular carcinoma	20a, 92a, 122, 618, 650
HTLV	Adult T-cell lymphoma	n/a
HPV	Cervical cancer	n/a
KSHV	Kaposi's sarcoma, Primary effusion lymphoma	17-92, 106b/25
HIV	Non-Hodgkin Lymphoma	223

*denotes viral-encoded miRNAs

miRNAs (BART6-5p, 6-3p, 14, 18-5p, 19-3p, and 2-5p) in serum that was significantly associated with nasopharyngeal carcinoma (NPC) diagnosis (11). Additionally, there was a positive correlation between the miRNA copy numbers detected in the tumor cells and those found in the serum. This suggests that changes seen in miRNA expression patterns in EBV positive tumors may also be potential biomarkers in serum samples. Furthermore, if future studies demonstrate that these miRNAs are a contributing factor in tumor development, they could act as a target for cancer treatment.

Hepatitis B and C infection in liver cancers

HBV and HCV are two distinct viruses associated with chronic liver disease and cancer. HBV is a DNA virus that infects approximately two billion people worldwide, resulting in over 350 million chronic infections and 600,000 deaths (12). In contrast, the single-stranded, positive-sense RNA virus HCV affects a smaller but still substantial percentage of the world's population with a prevalence of approximately 2% worldwide or 123 million people (13). Both infections are transmitted through blood products. Chronic infection with either virus can result in liver cirrhosis, a common risk factor for hepatocellular carcinoma (HCC) development. Recent evidence has detected an increase in mortality in cirrhosis-associated HCC cases, especially in patients infected with HCV (14). To determine which patients may be at risk for viral cirrhosis-associated liver cancer, studies have been conducted to investigate biomarkers correlated to HBV or HCV infection.

Evaluation of miRNA expression patterns in patient serum has revealed several specific markers that may be deregulated only in HCC patients infected with HBV or HCV. The most common miRNAs in HBV and HCV infected patients have been identified as mIR-21 (15), mIR-101 (16), miR-223 (15), miR-375 (17), and miR-20a, (18), miR-92a (18), miR-618 (19), miR-650 (19), respectively. miR-122 was identified as a potential biomarker in both HBV and HCV-related cancers. (20-22). Interestingly, miR-122 has also been shown to be important in HCV infection by facilitating viral replication and protecting the viral genome from degradation, suggesting multiple roles for these miRNAs in virus-cell interaction (23-24). In patients with HBV-related HCC, serum levels of miR-122 following surgery were significantly reduced, suggesting this marker may also have potential utility in measuring cancer progression and recurrence (20).

Knowledge of these virus associated miRNAs that are deregulated could contribute to disease detection and treatment. Detection of the miRNA in the serum itself may be a specific biomarker for early detection of the cancer. Furthermore, if this miRNA is deregulated in both the serum and tumor, it may indicate a potential pathway for target treated. mIR-21, which was shown to be upregulated in HBV-positive patients with HCC has been shown to modulated the PTEN pathway (25). Expression of certain miRNAs may also be used to evaluate treatment efficacy. Ji et al., (2009) reported an increased response to interferon treatment but lower overall survival in HCC patients with low miR-26 levels (26). Whether the miRNAs associated with viral expression could be utilized the same way has yet to be determined.

Human T-cell leukemia virus and adult T-cell leukemia and lymphoma

HTLV is a member of the retrovirus family that is aptly named because of its association with adult T-cell leukemia (ATL) and lymphoma. This RNA virus is estimated to infect 15-20 million people worldwide (27). Multiple groups have examined miRNA expression in HTLV infected cells, but reports examining miRNA expression profiles in serum could not be found. Cellular miRNAs associated with HTLV infection *in vivo* include miR-31 (28), miR-125b (29) and 9 other miRNAs identified *ex vivo* in cells from ATL patients (30). Future studies may determine if these miRNAs could also serve as serum biomarkers of HTLV associated cancer.

Human papillomaviruses

HPV is a DNA virus found in nearly 100% of cervical cancer cases (31). This virus has also been associated with oral, esophageal, and genital cancers. There are over 150 different HPV genomes variants classified that vary in phenotype and clinical presentation (32). miRNA analysis has been investigated in multiple studies of cervical tissue samples infected with HPV compared to normal controls (33-36). These papers detected a wide range of miRNA expression profiles associated with HPV infection and cancer with little overlap in results. The two miRNAs that were most frequently associated were miR-34a and miR-125b (37). While none of these studies examined serum, a large number of potential miRNAs have a potential association with HPV infection and cancer development.

Kaposi's sarcoma herpes virus

KSHV, also called HHV-8, is a DNA virus in the herpes virus family. KSHV malignancies depend on several factors including co-infection with HIV and state of the host immune response (38). KSHV not only regulates host miRNA expression but also encodes its own miRNAs. To date, 24 KSHV miRNAs have been reported (39). These viral miRNAs contribute to tumor development in virally infected cells.

In an elegant study analyzing miRNA expression associated with Kaposi's sarcoma and primary effusion lymphoma, two of the same miRNA clusters were identified as altered in both the cancer patients tumor and serum samples compared to normal controls (40). miRNAs in the 17-92 and 106b/25 clusters containing miRNAs 17, 18, 19a, 19b, 20a, 25, 92a, 93, 93#, and 106b were investigated. While the largest changes in miRNA expression were in tumor samples, increased expression of these clusters and miRNAs 17, 19a, 19b, and 92a were in patient plasma samples. Because of the large number of KSHV encoded miRNAs, as well as studies demonstrating their importance in tumor development, research involving KSHV associated cancer and miRNA expression is rapidly growing.

Human immunodeficiency virus and non-Hodgkin lymphoma

The HIV virus is an RNA virus of the *Retroviridae* family (41). Its primary cellular target is CD4+ T cells, macrophages, and dendritic cells. HIV infection is currently reported in approximately 35.3 million people worldwide (41). Patients with HIV infection have a 60-200 fold increased risk of developing non-Hodgkin lymphoma (NHL) and a 8-10 fold increased risk of developing Hodgkin lymphoma (42). Other types of lymphomas, as well as anal, cervical, hepatocellular, and lung cancers are also more prevalent in HIV-positive patients, especially in those co-infected with KSHV. Defining key markers of viral-induced oncogenesis is an important step in

early detection towards improving the outcome of these patients.

In a study examining patients with NHL, four miRNAs (miR-21, 122, 222, and 223) were identified as specific to virus infection, but only miR-222 demonstrated potential diagnostic utility as a serum marker of both HIV infection and NHL development (43). Upregulation of miR-21 was reported in circulating B-cells derived from NHL patients, although not in serum (44). This marker was also identified in the previously listed study of serum from HIV-positive NHL patients. While this method is only applicable to circulating cancer cells, it further demonstrates the potential utility of miRNA expression as a marker of virally related cancer progression.

Potential use of virus-associated miRNAs in cancer management

The discovery of miRNAs in serum has revealed a new potential route for evaluating biomarkers in cancers and other diseases (8). These biomarkers could potentially play a role in early detection of cancers, for monitoring cancer progression, or evaluation of effective treatments. Circulating miRNAs have potential clinical application due to the ease of testing serum samples (45). The largest virus-miRNA associations in serum studied to date involve miRNA detection in serum from HBV and HCV-associated cancers. These studies have reported miRNAs such as miR-122 which have altered expression in both primary tumors and in the serum in cancerous compared to normal controls and in relation to virus infection. Utility of miR-122 as both a biomarker and a therapeutic target in HCV-mediated liver cancer has been discussed (46). Identification of miRNA expression profiles in tumor tissues have multitudes of applications including understanding tumor development, improving treatment, and as reviewed in Figure 1, providing a potential tool for early cancer detection. Forthcoming studies will determine if miRNA serum profiles mimic those seen in cancerous tissue.

Multiple groups have investigated the use of serum miRNAs as biomarkers of cancer, metastases, Parkinson's disease, virus infection, heart disease, and some inflammatory conditions (47-49). Both serum and plasma could provide adequate samples for miRNA measurements, as they possess similar miRNA levels (50). It is

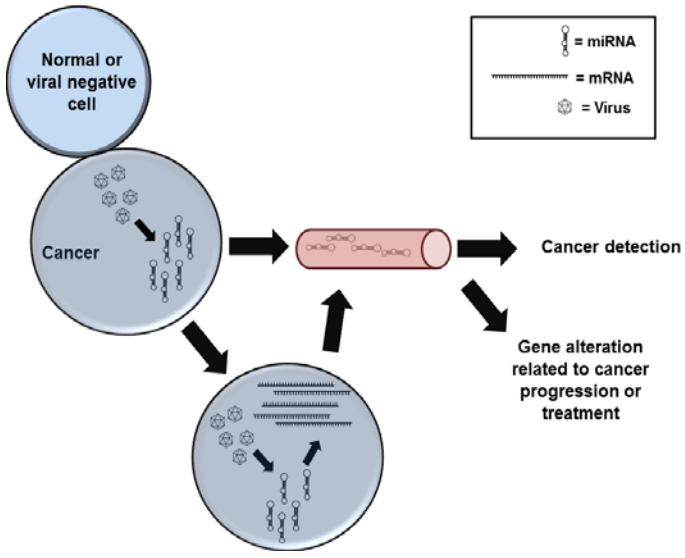

Figure 1: Summary of potential use of miRNAs in cancer management. Virus infection of cancer cells can alter miRNA expression. Changes in the same corresponding miRNA levels in serum samples could be used in early cancer detection and diagnosis. Changes in miRNA expression within cancer cells following viral infection may also alter cellular gene expression. If these genes impact cancer progression or cellular response to treatment, miRNAs in the serum may also be utilized in predicating cancer progression or response to treatment.

possible the circulating miRNAs detected in these studies are produced by cancer cells, as similar miRNA expression patterns have been reported in serum and tumors in both the EBV and KSHV studies reviewed here (11, 40). If further studies prove this to be true, many markers already identified as cancer-cell specific may be utilized as potential serum biomarkers.

Specific diagnostic tools are needed in virus related cancers. Many of the viruses reviewed here are prevalent at high frequencies in the human population, but only contribute to cancer under specific conditions. Improved diagnostic markers that can differentiate those susceptible to developing cancer following infection would have important clinical applications. Current virus diagnostic tools measure viral nucleic acids or proteins, or detect virus specific antibodies. While these can be useful in some cases, detection of the virus alone is not always able to distinguish individuals prone to cancer development. Additionally, if these circulating miRNAs are released from virus-positive cancer cells, they could be utilized as a marker of treatment efficacy and disease progression. These miRNAs may provide a novel, non-invasive method to distinguish between virally infected individuals and those susceptible to cancer development.

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