بسم الله الرحمن الرحيم

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شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

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Introduction

Hepatocellular carcinoma (HCC) is a leading cause of death worldwide **(*Tapper and Parikh, 2018*)**. It makes up 75%-85% of all primary liver cancers ***(Bray et al., 2018)*** and develops on a background of chronic liver disease, with hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcohol abuse and nonalcoholic fatty liver disease being the major etiologies ***(Craig et al., 2020)***. HCC is an aggressive malignancy and its diagnosis is usually late, with the survival rate about 6 to 20 months ***(Manghisi et al., 1998; El-Serag, 2011)***. Although the gold standard line of treatment is surgery, not all patients are eligible because of tumor stage or liver dysfunction. The lack of a curative pharmacological therapies for HCC, clarifies the utmost need for novel methods for better prognosis of HCC.

The basic cause of cancer is a group of dividing cells with high power and high resistance to the drugs that they are called cancer stem cells (CSCs) ***(Asghari et al., 2019)***. These CSCs are highly tumorigenic and are resistant to conventional lines of treatment ***(Liu et al., 2006; Alkatout et al., 2008)***. CSCs are now considered as the main cause for relapse of tumor as these cells are not affected by conventional therapies ***(Borovski et al.,***

2011). There are many types of CSC markers which varies according the organ of origin. CD90, found on CSC in liver cancer, may be an important prognostic marker and effective therapeutic target for the treatment of hepatic cancers ***(Yang et al., 2008)***. Thus, this marker can be used to identify potential hepatic CSCs from tumor specimens and blood samples of liver cancer patients **(*Hong et al., 2015*)**.

Angiogenesis is a crucial process in tumor pathogenesis as it sustains malignant cells with nutrients and oxygen. It is well known that tumor cells secrete various growth factors, including vascular endothelial growth factor (VEGF), which triggers endothelial cells to form new capillaries. Prevention of expansion of new blood vessel networks results in reduced tumor size and metastasis **(*Ambasta et al., 2011*)**. Angiogenesis inhibitors have thus become an important therapeutic approach in the treatment of HCC patients **(*Berretta et al., 2016*)**. Bevacizumab (Avastin), is a recombinant humanized immunoglobulin G (IgG) monoclonal antibody that targets VEGF-A and inhibits formation of the VEGF-A and vascular endothelial growth factor receptor-1 and 2 (VEGFR-1&2) complex thus restricting the tumor mass and reducing the possibility of metastases ***(Braghiroli et al., 2012)***. However, it is found to inhibit angiogenesis in normal cells as well. So it is

important to target VEGF in CSC using nanoparticle that encapsulates the drug/monoclonal antibody rendering them a targeted treatment for cancer only ***(Eskens and Verweij, 2006; Heddleston et al., 2010; d'Angelo et al., 2010)***. Therefore, we try to design a novel cancer therapy method that can be used to target angiogenesis near these cancer stem cells to prevent the recurrence of tumor.

The application of nanotechnology for the treatment of cancer is mostly based on early tumor detection and diagnosis by nanodevices capable of selective targeting and delivery of chemotherapeutic drugs to the specific tumor site **(*Singh et al., 2018*)**. The use of twin nanoparticle that binds specifically to targeted receptors and then unloads the drug on the liver malignant cells without affecting any other part of the body will thus result in decreasing the side effects and toxicity.

Super paramagnetic gold coated iron (Fe@Au) nanoparticles are promising candidates as magnetic drug carriers for tumor targeted drug delivery **(*Kayal and Ramanujan, 2010*)**.

Dumbbell-like nanoparticles (DBNPs) are referred to those with two different functional nanoparticles (NPs). NPs in intimate contact that offer an interesting platform for studying

physical and chemical properties of the materials based not only on each nanoparticle dimension and shape but also on the communication between the two NPs ***(Gu et al., 2005)***. Furthermore, the multi-functionality present within the DBNPs structure ensures that both magnetic and optical active NPs can be incorporated into one unit. The different NP surface also facilitates the controlled functionalization of each NP with the therapeutic drug. These advantages make the DBNPs a promising multi-functional probe for both diagnostic and therapeutic applications ***(Xu et al, 2008).***

Iron is tagged with VEGF monoclonal antibodies (MAb) and gold with MAb against cancer stem cell marker (CD90). Both iron and gold are stable and nontoxic. The use of external magnet can help the nanoparticle to invade to the interior parts of tumor and locate the cancer stem cells. Heat produced by this force of attraction can mediate release the VEGF monoclonal antibody to block endothelial cells near cancer stem cell ***(Ambasta et al., 2011)***. This strategy will inhibit angiogenesis near cancer stem cell hence new tumor cannot grow and old tumor will be unable to metastasize. This method can be very specific with very low toxicity in the body ***(Ambasta et al., 2011)***.

Aim of the Work

This study is designed to assess the therapeutic role of dumbell-like nanoparticles conjugated with monoclonal antibodies against both VEGF and cancer stem cell in hepatocellular carcinoma experimental model.

1. Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the dominant form of primary liver cancer; it develops in patients with chronic hepatitis in the advanced fibrotic or cirrhotic stage, and it is more than twice as common in men as in women ***(Villanueva, 2019)***.

1.1. Epidemiology

Liver cancer is the fifth most common cancer and the second most frequent cause of cancer-related death globally. HCC represents about 90% of primary liver cancers and constitutes a major global health problem **(*Galle et al., 2018*)**. Vaccination against hepatitis B virus (HBV) has resulted in a decrease in HCC prevalence **(*Marrero et al., 2018*)**. In Egypt, the prevalence of chronic hepatitis C infection is 13.8% of whole population and about 80% of the patients with hepatocellular carcinoma have underling hepatitis C **(*Demerdash et al., 2017*)**. Over the past 20 years, the incidence of HCC has become doubled from 2.6 to 5.2 per 100,000 populations ***(Bertuccio et al., 2017)***. According to European Association for The Study of The Liver in 2012, the pattern of HCC occurrence has a clear geographical distribution, with the highest incidence rates in East Asia, sub-Saharan Africa, and

Melanesia, where around 85% of cases occur. Several factors are known to be associated with a higher incidence of HCC: male gender, increasing age, environmental and geographic factors, metabolic and genetic factors (e.g., non-alcoholic steatohepatitis (NASH), genetic hemochromatosis), viral infection, alcohol intake, oncogenic factors (e.g., aflatoxin), and histological stage ***(Puoti, 2018)***.

Preexisting cirrhosis is found in more than 80% of individuals diagnosed with HCC ***(Bruix and Sherman, 2011)***. The major causes of cirrhosis, and hence HCC, are HBV, hepatitis C virus (HCV), alcohol, and nonalcoholic fatty liver disease (NAFLD), but less-prevalent conditions, such as hereditary hemochromatosis, primary biliary cholangitis (PBC), and Wilson's disease, have also been associated with HCC development ***(White et al., 2012)***. Human immunodeficiency virus (HIV) infected individuals have been suggested to have higher risk of developing HCC **(*Merchante et al., 2013*)**.

1.2. Pathogenesis

The development and progression of HCC is a multistage process. A chronic insult (e.g., HCV, HBV, and alcohol) induces liver injury through reactive oxygen species (ROS) production, cellular DNA damage, endoplasmic

reticulum stress, and necrosis of damaged hepatocytes. Most HCCs arise in the setting of chronic hepatitis induced by HCV or HBV infection **(*Hernandez-Gea et al., 2013*)**. HCV is a single stranded RNA virus that cannot integrate into the host genome but triggers an immune-mediated inflammatory response that promotes neoplastic transformation of damaged hepatocytes ***(Levrero, 2006)***. Conversely, HBV can integrate into the genome of infected hepatocytes and promotes hepatocarcinogenesis through sustained inflammatory damage, hepatocyte regeneration, direct oncogenic transformation following integration of the viral genome into host genes, and the transactivating potential of several viral oncoproteins. The sustained dysregulation of the liver cell by HBV infection can ultimately affect DNA repair mechanisms and promote mutational events, which contribute to malignant transformation of hepatocytes **(*Hernandez-Gea et al., 2013*)**. The development of dysplastic nodules and their malignant transformation to early HCC discussed in Figure (1) ***(Seitz and Stickel, 2006)***.

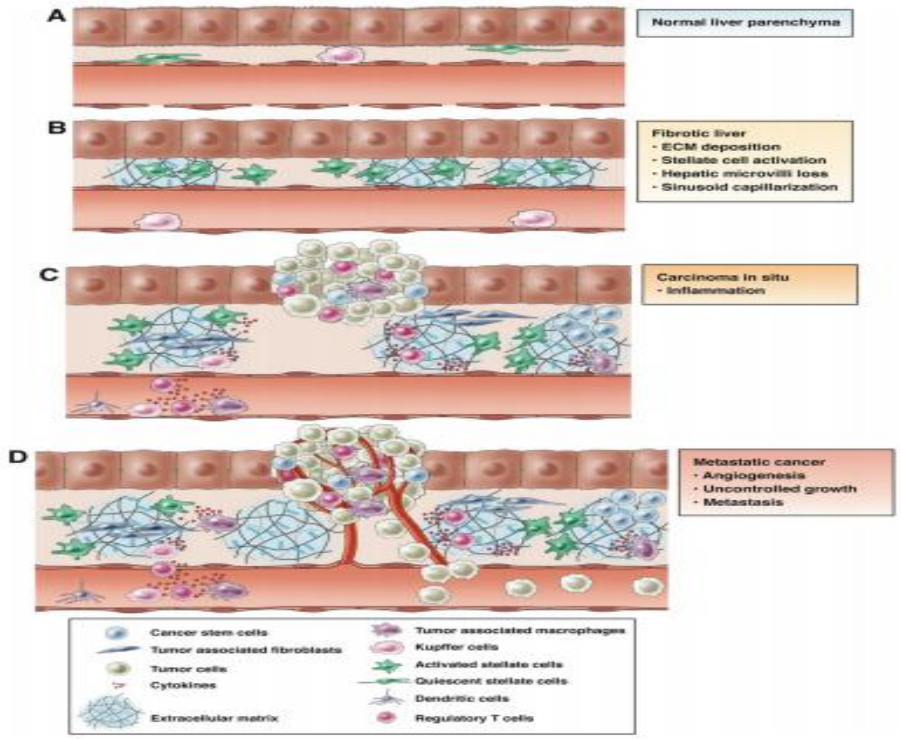

Figure (1): Anatomic and cellular alterations leading to the development of HCC. (A) Normal liver parenchyma. Hepatocytes with microvilli and sinusoidal endothelial cells whose fenestrations favor metabolic exchange. Space of Disse with few quiescent stellate cells containing lipid droplets. (B) Fibrotic liver. Upon chronic liver injury, hepatocytes lose their microvilli, sinusoid endothelial cells become defenestrated, and stellate cells are activated, losing lipid droplets and secreting extracellular matrix (ECM). (C) HCC Malignant transformation of hepatocytes with uncontrolled growth. Infiltration of inflammatory cells and cytokines with extensive fibrosis and recruitment of tumor associated fibroblasts (TAFs) and cancer stem cells (CSCs). (D) Development of new vessels (neoangiogenesis) and distant metastases **(*Hernandez-Gea et al., 2013*)**.

1.2.1. Caspase and apoptosis

Diverse stimuli can trigger apoptosis from inside or outside the cell, for example, contradictory cell cycle or developmental death signals, cell surface receptors, DNA damage, cytotoxic drugs, and irradiation ***(Runyan et al., 2006; Kulik et al., 2008)***. Inflammatory cytokines (e.g., TNFα) can continually induce the activation of caspase-8, caspase-3, and DNA fragmentation through membrane receptors ***(Jaeschke et al., 2000)***. Being a highly selective process, apoptosis is important in both physiological and pathological conditions ***(Merkle, 2009; Mohan, 2010)***. Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic (or mitochondrial) and extrinsic (or death receptor) pathways of apoptosis. Both pathways eventually lead to a common pathway of apoptosis. A third less well-known initiation pathway is the intrinsic endoplasmic reticulum pathway ***(O'Brien and Kirby, 2008)***. The common pathway of apoptosis involves the activation of a series of caspases. The upstream caspase for the intrinsic pathway is caspase 9 while that of the extrinsic pathway is caspase 8. The intrinsic and extrinsic pathways converge to caspase 3. Caspase 3 then

cleaves the inhibitor of the caspase-activated deoxyribonuclease, which is responsible for nuclear apoptosis ***(Ghobrial et al., 2005)***. Reduced apoptosis or its resistance plays a vital role in carcinogenesis. Generally, the mechanisms by which evasion of apoptosis occurs can be divided into: 1) disrupted balance of pro-apoptotic and anti-apoptotic proteins, 2) reduced caspase function and 3) impaired death receptor signaling. Figure 2 summarizes the mechanisms that contribute to evasion of apoptosis and carcinogenesis ***(Wong, 2011)***. The caspases can be broadly classified into two groups: 1) those related to caspase 1 (e.g. caspase-1, -4, -5, -13, and -14) and are mainly involved in cytokine processing during inflammatory processes and 2) those that play a central role in apoptosis (e.g. caspase-2, -3. -6, -7, -8, -9 and -10). The second group can be further classified into initiator caspases (e.g. caspase-2, -8, -9 and -10) which are primarily responsible for the initiation of the apoptotic pathway and effector caspases (caspase-3, -6 and -7) which are responsible in the actual cleavage of cellular components during apoptosis ***(Fink and Cookson, 2005)***. Dysregulation of apoptotic signaling can cause insufficient apoptosis leading to cancer (cell accumulation, resistance to therapy, defective tumor surveillance by the immune system), persistent infections (failure to eradicate infected cells) ***(Patel et***

al., 1998; Chabot et al., 2013). Tumorigenesis is not merely the result of excessive proliferation due to the activation of oncogenes, but also frequent impairment of apoptosis checkpoints ***(Wang, 1999; Wen et al., 2012)***.

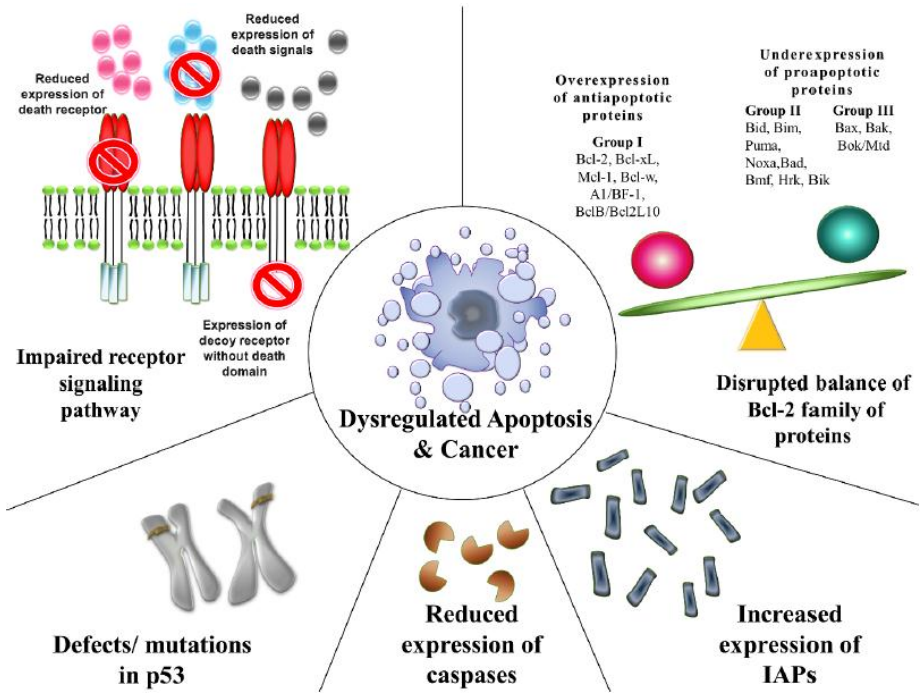

Figure (2): Mechanisms contributing to evasion of apoptosis and carcinogenesis ***(Wong, 2011)***.

1.2.2. Angiogenesis and its role in HCC

Angiogenesis plays an important role in hepatocarcinogenesis from its early stages **(*Zhu et al., 2011*)**. HCC is a highly vascularized tumor; indeed, pathological angiogenesis is one of the main contributors to chronic liver diseases. The hepatic wound-healing response due to chronic