Mesenchymal Stem Cells in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths worldwide and it is major health problem despite the emergence of different preventive and treatment modalities. It is urgent to find a safe and effective therapy for the HCC treatment. Mesenchymal stem cells (MSCs) therapy has been discussed as an attractive novel therapeutic option for liver diseases and cancer. In this review We analysed human umblical cord blood MSCs (huc MSCs) as they are hypothesized to be potent candidates for the clinical application of allogenic MSC-based therapies as they can easily be isolated from umbilical cord blood, cultured or modified in vitro, Although controversy exists and stem cell-based therapy will not completely take place of the conventional therapeutic tools, researchers still hold the view that cancer therapy based on huc-MSCs has a promising future. Nevertheless, it should be cautious when using huc-MSC as therapeutic vehicles for HCC treatment as the effects of MSCs on tumor progression remain controversial and no consistent conclusion has been presented until now. Thus, the effects of the MSCs from different sources on HCC need more data about disclose the molecular mechanism of MSCs migration to improve recruitment efficiency and the effectiveness of targeted therapy and whether MSCs functions are in correlation with MSCs sources or HCC subtypes or research methodology

Keywords: Hepatocellular carcinoma, mesenchymal stem cells, human umblical cord blood mesenchymal stem cells, cancer therapy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer, the most common malignant primary tumor of the liver and the third leading cause of cancer-associated mortality worldwide (1).

The pathogenesis of HCC is related to multiple risk factors, including chronic hepatitis B, hepatitis C virus infection, alcohol consumption, non-alcoholic fatty liver disease and aflatoxin B1 intake. These factors may induce DNA damage, epigenetic alterations and cancer-related mutations (2).

Despite continuous advancements in the management of HCC, the prognosis remains inferior compared to other tumor entities as HCC cases are diagnosed during advanced stages. Surgical interference remains the standard treatment options for HCC. Unfortunately, the surgical option is less effective during advanced stages of HCC (3).

Mesenchymal stem cells (MSCs) are non-hematopoietic multipotent stem cells with the ability to differentiate into mesodermal lineage (osteocytes), ectodermal lineage (neurocytes) and endodermal lineage (hepatocytes). MSCs can be obtained from various tissues, such as bone marrow, adipose tissue, and umbilical cord blood. Human umbilical cord-derived (hUC)-MSCs are a superior choice over the other types of MSCs as no ethical challenges, low risk of viral transmission, low immunogenicity, readily available and more primitive (4).

MSCs have been applied as a potentially promising cell therapy approach for cancer and for treating liver diseases via tissue repair and immune regulation. MSC-secreted extracellular vesicles including microvesicles and exosomes may contribute to the therapeutic potency of MSCs by mediating cell–cell micro-communication and transporting paracrine factors during angiogenesis, tissue regeneration and immune regulation (5).

Aim of the study was the tumor suppressive effects of MSCs in HCC

Hepatocellular carcinoma

HCC is the most common primary liver cancer and accounts for ~90% of cases. HCC is the fifth most common cause of cancer mortality and the fourth common cancer in Egypt, Egypt ranks the third and 15th most populous country in Africa and worldwide, respectively and it has a tremendous influence on the socioeconomic development (6).

The major risk factors for liver cancer include viral hepatitis infection, food additives, alcohol abuse, toxic exposure (aflatoxin-B1 (AFB1) intake from contaminated food), environmental and industrial toxic chemicals, and air and water pollutants. The clinical history of approximately 80% of HCC patients progresses from fibrosis to cirrhosis and finally to cancer (7).

In contrast to most solid cancers, the incidence of HCC and HCC-related deaths have increased over the last several decades in many parts of the world and the prognosis of HCC is dismal with a 3-year survival rate of 12.7% and a median survival of 9 months (8).

Despite recent advances in the prevention, surveillance, diagnosis, treatment and multidisciplinary collaboration of HCC, only 40% of HCC cases are diagnosed at an early stage nationally and it remains highly lethal. Death among HCC patients occurs mainly due to tumor

progression, with recurrence and metastasis, even after curative treatments at the early stage. For HCC, delays as little as 3 months in diagnostic follow-up can allow for significant tumor growth and lead to lower chances of effective treatment options. A better understanding of breakdowns in follow-up is necessary to identify appropriate intervention strategies **(9).**

Chronic liver disease and cirrhosis remain the most important risk factors for the development of HCC of which viral hepatitis and excessive alcohol intake are the leading risk factors worldwide. Hepatitis B and C are the most common causes of chronic hepatitis in the world. Hepatitis B carriers have a 10%–25% lifetime risk of developing HCC. Unlike other causes of chronic hepatitis, HBV is unique in that HCC can develop without evidence of cirrhosis. The hepatocarcinogenicity of HBV can be significantly reduced as the use of HBV vaccination **(10).**

In hepatitis C, the development of HCC occurs almost exclusively in the liver with established cirrhosis; Dual infection with HBV and HCV in a cirrhotic patient increases the risk of HCC. The risk of HCC is reduced significantly in patients who obtained a sustained viral response after treatment of HCV with a 54% reduction in all-cause mortality. Other predisposing conditions as sex may play a role in the development of HCC. HCC occurs more often in males, with a ratio of 2:1–4:1; however, this may not be due to sex alone **(11).**

Aflatoxin produced by Aspergillus species (molds) found on grains, corn, peanuts, or soybeans stored in warm humid conditions is a potent hepatocarcinogen. The risk of HCC with aflatoxin is dependent on the dose and duration of exposure. Metabolic and genetic diseases associated with HCC include hemochromatosis, Wilson's disease, α-1 antitrypsin disease, tyrosinemia, glycogen-storage disease types I and II, and porphyries. Other risk factors may include smoking. Cigarette smoking is associated with a significant increase in the development of HCC **(12).**

The hallmark imaging features of HCC are arterial phase hyperenhancement (APHE) and portal/delayed washout, which represent the characteristic vascular profile of HCC on dynamic CT or MRI. However, approximately 40% of HCCs do not demonstrate these imaging findings, resulting in a low sensitivity of imaging tests for the diagnosis of HCC **(13).**

Novel diagnostic and/or prognostic biomarkers are emerging as key players in the field of HCC research. For example, annexin A2, surviving and alpha-1 antitrypsin (**14).**

Likewise, comprehensive genomic and epigenomic approaches are emerging as promising tools to stratify HCCs into clinically relevant subgroups. Pathologically, the overall poor outcome of HCCs expressing stem cell markers such as keratin 19 (K19), epithelial cell adhesion molecule (EpCAM), and CD133 has been reported, potentially via hypoxia-induced epithelial to mesenchymal transition (**15).**

MSCS

Stem cells can be defined as units of organization of biological systems that are responsible for the regeneration and development of organs and tissues. These cells can also be considered as units of evolution via natural selection. Stem cells are undifferentiated cells with clonogenic potential that are capable of self-renewal and differentiation into multiple cell lineages. This is achieved by either symmetrical or asymmetrical cell divisions (16).

In symmetrical cell division, a cell divides to produce two identical cells; this kind of division is common in early stages of development (17).

On the other hand, in asymmetrical cell divisions, a cell divides into two cells, one of them being identical to the original cell and the other being a different type of cell. This kind of division maintains the pool of stem cells whilst producing more differentiated tissue specific cells, and is dominant in adult tissues (18).

Sources and potency of stem cells

There are four main sources of these cells, namely embryonic tissue, fetal tissues (i.e., directly the fetus or extrafetal tissues), adult tissues (e.g. fat, bone marrow, blood, skin or skeletal muscle), and differentiated somatic cells after they have been genetically reprogrammed, i.e., induced pluripotent stem cells (iPSCs) (19)

Embryonic stem cells derived from blastocyst, i.e. a later stage of embryonic development, are pluripotent, i.e. they can create any tissue in the body except the placenta. Cells derived directly from fetus are usually multipotent, i.e. able to differentiate only to a limited number of specialized cell types. However, pluripotent cells can typically be obtained from extrafetal tissues, particularly amnion, amniotic fluid, chorion and umbilical cord while cells from umbilical blood were rather multipotent (20).

Adult stem cells are located in practically all organs and tissues of the adult organism, e.g. skin, brain, heart, blood vessels, skeletal muscle, intestine, liver, kidneys, reproductive organs, adipose tissue and bone marrow, and they are also in body fluids, such as blood (including menstrual blood and urine (21).

Adult stem cells are usually multipotent. However, some adult stem cells are oligopotent, bipotent or even unipotent, e.g. basal cells in the epidermis, spermatogonial stem cells and satellite cells in skeletal muscles (22).

Mesenchymal stem cells (MSCs): MSCs are multipotent adult progenitor cells capable of differentiating into various mesenchymal tissues, were first discovered in bone marrow (BM) as fibroblastic colony-forming units due to their multipotency and paracrine effect, and later confirmed to be isolated from a variety of human tissues, such as adipose tissue, nervous tissue, umbilical cord and amniotic fluid (23).

MSCs are ideal candidates for regenerative medicine. Currently, there is no consensus on a single surface molecule to identify MSCs from various sources. The minimum criteria of MSCs include: (a) remain plastic-adherent under standard culture conditions; (b) express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR; (c) differentiate into osteoblasts, adipocytes, and chondrocytes in vitro. Other surface antigens generally expressed by MSCs include CD13, CD29, CD44, and CD10 (24).

Use of adult BM MSCs brought to the forefront, but limits their clinical application as they are extremely low while there is a high possibility of virus contamination. Moreover the number of stem cells, the ability to expand, differentiate significantly decreased with age and cease to divide in vitro by passage 6–10 (25)

Adipose tissue MSCs (AD MSCs) would be a useful alternative to BM MSCs for autologous grafting in aged individuals. Unfortunately AD MSCs are rarer than BM MSCs. Therefore, extraction and expansion may be required prior to therapeutic use. (26).

Mechanisms of MSC homing

MSCs can home to sites of damaged tissue, which is the premise of their application in the treatment of systemic diseases. Considerable evidence from numerous studies indicated that exogenous MSCs transplanted into the human body were preferentially captured by the vascular system of the target tissue and then migrated to the target tissue across vascular endothelial cells (27).

Particularly, ischemic-damaged tissues can attract MSCs that can home to damaged tissues where they play a therapeutic role. However, unlike the process of leukocyte migration to inflammatory sites, the mechanism of MSC homing is not well understood. In general, MSC homing can be divided into non-systemic homing and systematic homing (28).

Non-systemic homing refers to local transplantation of MSCs to the injured site while systemic homing of MSCs is guided by homing-promoting factors released from damaged or inflamed tissues, which is similar to the migration of circulating leukocytes to inflammatory sites and is categorized into five consecutive steps: (1) rolling, (2) activation, (3) firm adhesion, (4) crawling, and (5) transendothelial migration (29).

Delivery of mesenchymal stem cell

Intravenous injection, in contrast to the intraperitoneal and intrahepatic injection of MSCs, provides the most effective treatment as MSCs in the venous blood can stimulate IL10 release, which, in turn, can modulate the host immune response (30).

Umbilical cord blood stem cells are cells of choice: Umbilical cord blood offers a very good source of stem cells for both research and clinical advantages over other sources of stem cells. Moving toward effective clinical applications requires a readily abundant supply of stem cells to

provide the needed amounts of stem cells. With raising the global birth rate, umbilical cord blood can be considered as one of the most abundant sources of stem cells. Collection of huc MSC is easier, non-invasive, pain-free, lower risk of infection transmission and less expensive **(31).**

Hematopoietic Stem Cells (HSCs): The majority of haematopoietic stem cells are found in the fetal circulation, in the placenta and umbilical cord. The haematopoietic stem cells migrate to the bone marrow within hours after delivery, where they take up residence and provide a life-long supply of stem cells and progenitors of all the blood-forming elements, including erythrocytes, leucocytes and platelets. Stem cells remain in the placenta and umbilical cord post-delivery and on average 120 ml of blood can be collected with no risk to either the mother or the baby. Furthermore, this rich source of stem cells is usually discarded **(32).**

Multipotent non-hematopoietic stem cells: These stem cells are small in size and exist at low density in cord blood and are negative for the major hematopoietic marker CD45. This population of cells has been shown to express transcription factors normally expressed by embryonic stem cells **(33).**

The term "embryonic-like" was given to the cells based on their expression of so-called embryonic stem cell markers such as OCT4, the main pluripotency key player in embryonic stem cells. It has been shown that OCT4 has two splice variants OCT4A and OCT4B that differ only in their N terminals whilst having identical C terminals. The splice variants have been shown to have different temporal and spatial expression patterns. Whilst OCT4A was expressed mainly in the nuclei of human embryonic stem cells, OCT4B was detected in many different types of differentiated cells **(33).**

Umbilical cord blood Mesenchymal Stem Cells (UC-MSCs): UC-MSCs are a class of multifunctional stem cells isolated and cultured from umbilical cord. They possessed the characteristics of highly self-renewal, low immunogenicity and multi-directional differentiation potential into adipocytes, osteocytes, chondrocytes, neurons and hepatocytes, although some differentiation abilities are known to be partial. Its application in the field of tissue engineering and gene therapy has achieved a series of results **(34).** UC-MSCs can be isolated from UC blood, perivascular areas, amniotic fluid, and Wharton's Jelly (WJ) figure **1 (35).**

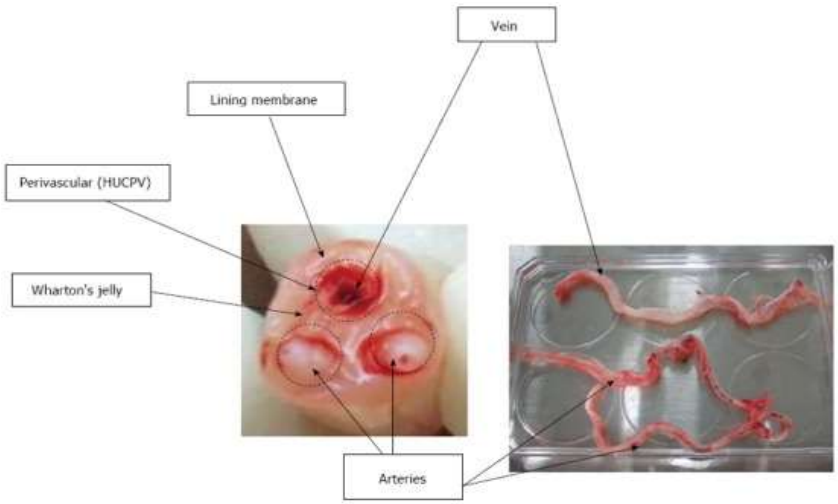

Figure (1): Various compartments of umbilical cord from which mesenchymal stem cells can be isolated. HUCPV: Human umbilical cord perivascular **(35).**

Cord blood derived MSCs show high morphological and molecular similarities to bone marrow derived MSCs including the lacking of hematopoietic surface antigens CD133, CD34 and CD45. UC blood MSCs demonstrate higher proliferation capabilities than BM MSCs. UC blood MSCs have been successfully differentiated in-vitro into osteogenic, chondrogenic, neural and hepatic lineages **(36)**

Immunological Properties of UC-MSCs

Huc-MSCs did not stimulate the proliferation lymphocytes and did not induce allogeneic or xenogeneic immune cell responses. Huc-MSCs produced an immunosuppressive isoform of HLA-I, and did not express HLA-DR. Flow cytometry revealed that the expression of immune response-related surface antigens, such as CD40, CD40L, CD80 and CD86 was absent on the huc-MSCs. These results suggest that huc-MSCs may be tolerated in an allogeneic transplant. **(37).**

Proliferation and Differentiation Potential of UC-MSCs

The Mennan team's report indicated that there was no significant difference in proliferation rate no matter what compartment UC-MSCs come from, and the average doubling time of UC-MSCs between P0 and P3 was 2–3 days, significantly faster than BM-MSCs **(38).**

UC-MSCs have the ability of multi-directional differentiation and have potential to differentiate into bone, adipose, cartilage and other tissues, thus they can be used to repair various tissues and organs, and are ideal seed cells in the field of regenerative medicine **(39).**

Huc-MSCs are used in the treatment of various diseases

They have several distinct properties essential for their therapeutic applications. (1) Differentiation: the generation of differentiated cells by Huc-MSCs promotes tissue regeneration

and improves tissue function. (2) Immune regulation: Huc-MSCs inhibit the proliferation of immune cells, such as T cells, B cells, cells; induce the differentiation of macrophages from pro-inflammatory phenotypes to anti-inflammatory phenotypes; and reduce inflammation by secreting interleukin-10 (IL-10) and interleukin-4 (IL-4). (3) Paracrine effects: Huc-MSCs promote tissue regeneration by secreting soluble molecules such as keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and other cytokines **(40)**. (4) Anti-inflammatory effect: Huc-MSCs suppress the secretion of inflammatory factor interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8), reducing inflammation and oxidative stress, thus suppressing cell apoptosis **(41).** (5) Anti-fibrotic activity: Huc-MSCs stimulate fibrosis-related cell apoptosis and the secretion of HGF and other molecules. The anti-fibrotic function can also be mediated by the regulation of related signaling pathways and the promotion of vascular remodeling. (6) Non-coding RNA regulation: Huc-MSCs can affect the expression of microRNA (miRNA), long non-coding (lncRNA), and circular RNA (circRNA), indirectly regulating their target genes and achieving therapeutic effects **(42).**

Therapeutic effects of different generations of Huc-MSCs

The therapeutic effects of Huc-MSCs are dependent on the number of their generations in culture, Huc-MSCs at less than 10 passages have better cardiogenic differentiation ability, while at passages 11–20, the differentiation towards nerve cells is evidently enhanced **(43).**

In the treatment of acute liver failure, Huc-MSCs harvested at different passages show distinct effects. Cells at passage 5 are more potent than passage 10 cells in homing to the liver, as well as in enhancing proliferation and inhibiting apoptosis of liver cells **(44).**

These findings imply that cells at different passages may have distinct therapeutic effects in various diseases, and selection of an appropriate passage of Huc-MSCs according to the type of disease may be required to achieve optimal therapeutic results. However, no conclusive evidence for the relationship between the number of cell passages and disease treatment effects is currently available **(45)**

Mechanism of Huc-MSCs in treating liver disease

Primary hepatocyte transplantation can be used as an alternative method to liver transplantation. Transplanted hepatocytes proliferate to regenerate the damaged liver and compensate for the loss of liver function. However, the practical application of hepatocyte transplantation is restricted by the availability of donor cells and their limited proliferative potential in vitro. Research on regenerative medicine and stem cells has rapidly advanced in recent years **(46).**

Significant advances have been achieved in using MSCs to treat liver disease, both in preclinical and clinical trials. Many studies have shown that MSCs can play a therapeutic role in treating liver diseases through various mechanisms: (1) inhibiting hepatocyte apoptosis and promoting

hepatocyte regeneration, (2) para-secretion of a variety of cytokines to synergistically protect against liver fibrosis, and (3) regulating immunity to reduce the inflammatory response and restore the steady state of the body. Multiple clinical trials have verified the therapeutic efficacy of MSCs in liver diseases **(29)**

Tumorigenicity of Huc-MSCs

The effects of MSCs on tumor cells remain controversial. Li et al. demonstrated that MSCs enhanced tumor growth but significantly inhibited the invasiveness and metastasis of HCC and MSC could be useful in controlling metastatic recurrence of HCC **(47).**

The tumorigenicity of transplanted Huc-MSCs, which will affect the therapeutic effect, is one of the current concerns. The experiments on tumorigenicity safety conducted by Jun-Won Yun and coworkers in mice did not detect tumors related to Huc-MSC **(48).**

Huc-MSCs can exert an anti-tumor effect also by affecting transcriptional regulation in leukemia cells. Additionally, Huc-MSCs can enhance the proliferation and migration of cancer cells So far, there is no conclusive proof of Huc-MSC tumorigenicity or their ability to promote the development of cancer already present in the organism **(49).**

Huc-MSCs and HCC

Recently, massive research has been devoted to exploring the association between HCC and MSCs. The function of MSCs in the occurrence, development and treatment of HCC is quite controversial. A growing body of research illustrated that MSCs have the dual characteristics of suppressing and promoting tumors through different molecular signaling mechanisms. Even in different stages of HCC, MSCs also play a contradictory role. In the early stage of HCC, they can reduce DNA damage and ROS accumulation to play a tumor suppressor effect. However, in the late stage, MSCs manifested as a tumor promoter on HCC by promoting the stem cell-like properties and epithelial-mesenchymal transition **(50).**

The anti-tumor mechanism of UC-MSCs

The inhibitory effect of UCMSCs in the initiation and development of HCC has been demonstrated consecutively. Liu et al. described the inhibition effect of UCMSCs on the growth, migration, metastasis and angiogenesis of HCC cells **(51).**

Exosomes derived from hUC-MSCs reduce the oxidative stress level of liver tumors, thereby suppressing the acute liver injury and fibrosis induced by CCl4 and the growth of liver tumors, and significantly reducing tumor size, inflammation and infiltration area **(52).**

UCMSCs may restrain the growth of co-cultured hepatocarcinoma cells by down-regulating AFP, Bcl-2 and Survivin, and accelerate cell apoptosis which is related to the apoptosis signal

pathway. Furthermore, UCMSCs function in a time-dependent and cell-number-dependent manner (53).

Accumulating studies with in vitro experiments and animal models have shown the ability of MSCs homing to tumor microenvironment. The migration capacity of MSCs is raised by highly tumorigenic HCC cells, which makes MSCs an ideal carrier for targeted therapy of liver cancer. Compared with anti-tumor monotherapy, the superiorities of synergistic combination therapy are including promoting the efficiency and specificity of MSCs-mediated anti-cancer, reversing drug resistance, improving radiotherapy potential and safety (54)

Yet, in spite of considerable research data highlight the prospect of MSCs and their secreted exosomes be modified or directly used to treat HCC but the two-way effect of MSCs on tumor vicious process still should not be ignored. When MSCs are used for treatment, the possibility that they may induce tumor recurrence exists (55).

Furthermore, soluble factors in conditioned medium (CM) derived from MSCs promote anti-oxidant, immunosuppressive and cell proliferative effects at the transplant regions. The CM has both differentiation capacity and therapeutic function. Thus, it can be able to differentiate the cells into different lineages and the paracrine effect of these cells helps in replacement of the damaged cells. This derived CM might intensify the positive effects of cell-based therapies (56).

Conclusion

Huc-MSCs have shown more promising spotlight for achieving more acceptable outcomes in HCC. Although controversy exists and stem cell-based therapy will not completely take place of the conventional therapeutic tools, researchers still hold the view that cancer therapy based on huc-MSCs has a promising future. Nevertheless, it should be cautious when using huc-MSC as therapeutic vehicles for HCC treatment as the effects of MSCs on tumor progression remain controversial and no consistent conclusion has been presented until now. Thus, the effects of the MSCs from different sources on HCC need more data need to be acquired on whether MSCs functions are in correlation with MSCs sources or HCC subtypes or research methodology. Therefore, the in-depth mechanisms underlying the protective effects of MSCs require further investigation. Clarification of the predominant mechanisms in different situations will improve the safety, efficacy and outcomes of MSC-based therapy.

References

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level. JAMA Oncol. 2017; 3:1683–91.
2. Huang Z, Zhou JK, Peng Y, He W and Huang C. The role of long noncoding RNAs in hepatocellular carcinoma. Mol Cancer. 2020; 19 (1):77.

3. Tsilimigras DI, Bagante F, Sahara K, Moris D, Hyer JM. Prognosis After Resection of Barcelona Clinic Liver Cancer (BCLC) Stage 0, A, and B Hepatocellular Carcinoma: A Comprehensive Assessment of the Current BCLC Classification. Ann Surg Oncol. 2019; 26(11):3693-3700.
4. Yin F, Wang WY and Jiang WH. Human umbilical cord mesenchymal stem cells ameliorate liver fibrosis in vitro and in vivo: From biological characteristics to therapeutic mechanisms. World J Stem Cells. 2019;11 (8):548-564.
5. Lou G, Chen Z, Zheng M and Liu Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med. 2017; 49(6):e346.
6. Rashed WM, Kandeil MAM, Mahmoud MO, Ezzat S. Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. J Egypt Natl Canc Inst. 2020 Jan 16;32(1):5.
7. Arboatti AS, Lambertucci F, Sedlmeier MG, Pisani G, Monti J, Álvarez ML, et al. Diethylnitrosamine Increases Proliferation in Early Stages of Hepatic Carcinogenesis in Insulin-Treated Type 1 Diabetic Mice. Biomed Res Int. 2018 Apr 23; 9472939.
8. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66:7-30.
9. Kubota K, Ina H, Okada Y, Irie T. Growth rate of primary single hepatocellular carcinoma: determining optimal screening interval with contrast enhanced computed tomography. Dig Dis Sci. 2003; 48:581–586.
10. Chiang CJ, Yang YW, You SL, Lai MS, Chen CJ. Thirty-Year Outcomes of the National Hepatitis B Immunization Program in Taiwan. JAMA, 2013; 310(9):974–6
11. Zhang X, Guan L, Tian H, Zeng Z, Chen J, Huang D, Sun J, Guo J, Cui H, Li Y. Risk Factors and Prevention of Viral Hepatitis-Related Hepatocellular Carcinoma. Front Oncol. 2021 Sep 9;11:686962.
12. Magnussen A, Parsi MA. Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol. 2013 Mar 14; 19(10):1508-12.
13. Yoon, S.H, Lee, J.M, So, Y.H, Hong, S.H, Kim, S.J, Han, J.K, et al. Multiphasic MDCT Enhancement Pattern of Hepatocellular Carcinoma Smaller Than 3 cm in Diameter: Tumor Size and Cellular Differentiation. Am. J. Roentgenol. 2009, 193, W482–W489
14. Shimada S, Mogushi K, Akiyama Y, Furuyama T, Watanabe S, Ogura T, et al. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. EBioMedicine 2019; 40: 457-470
15. El Jabbour T, Lagana SM, Lee H. Update on hepatocellular carcinoma: Pathologists' review. World J Gastroenterol. 2019 Apr 14;25(14):1653-1665.
16. Yamashita, Y. M, Fuller, M. T, Jones, D. L. Signaling in stem cell niches: lessons from the Drosophila germline. J. Cell Sci, 2005; 118: 665.
17. Shahriyari L and Komarova, N L. Symmetric vs. asymmetric stem cell divisions: an adaptation against cancer? PLoS One, 2013; 8: 76195.
18. Verfaillie C. Pluripotent stem cells. Transfus Clin Biol, 2009; 16: 65-9.

19. Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, et al. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. Biotechnol Adv. 2018 Jul-Aug;36(4):1111-1126
20. Jaing TH. Umbilical cord blood: a trustworthy source of multipotent stem cells for regenerative medicine.Cell Transplant, 2014; 23: 493-496
21. Schosserer M, Reynoso R, Wally V, Jug B, Kantner V, Weilner S, et al. Urine is a novel source of autologous mesenchymal stem cells for patients with epidermolysis bullosa. BMC Res. Notes, 2015; 8: 767
22. Visvader JE, Clevers H. Tissue-specific designs of stem cell hierarchies. Nat. Cell Biol, 18 (2016), pp. 349-355
23. Kobolak, J, Dinnyes, A, Memic, A, Khademhosseini, A, Mobasheri, A. Mesenchymal stem cells: identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods, 2016; 99: 62–68
24. Buhring HJ, Battula VL, Treml S. Novel markers for the prospective isolation of human MSC. Ann N Y Acad Sci 2007;1106: 262–271
25. Kern, S, Eichler, H, Stoeve, J, Klüter, H, Bieback, K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells, 2006; 24, 1294–1301.
26. Götherström C, West A, Liden J, Uzunel M, Lahesmaa R, Le Blanc K. Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells. Haematologica. 2005 Aug; 90(8):1017-26
27. Li X, Wang Q, Ding L, Wang YX, Zhao ZD, Mao N, et al. Intercellular adhesion molecule-1 enhances the therapeutic effects of MSCs in a dextran sulfate sodium-induced colitis models by promoting MSCs homing to murine colons and spleens. Stem Cell Res Ther. 2019; 10:267.
28. Nitzsche F, Muller C, Lukomska B, Jolkkonen J, Deten A, Boltze J. Concise review: MSC adhesion cascade-insights into homing and transendothelial migration. Stem Cells. 2017; 35: 1446–60.
29. Yuan M, Hu X, Yao L, Jiang Y, Li L. Mesenchymal stem cell homing to improve therapeutic efficacy in liver disease. Stem Cell Res Ther. 2022 May 3; 13(1):179.
30. Zhao W, Li JJ, Cao DY, Li X, Zhang LY, He Y, et al. Intravenous injection of mesenchymal stem cells is effective in treating liver fibrosis. World J Gastroenterol. 2012 Mar 14; 18(10):1048-58.
31. Mcguckin CP, Forraz N. Potential for access to embryonic-like cells from human umbilical cord blood. Cell Prolif, 2008; 41 Suppl 1: 31-40.
32. Rogers I, Casper RF. Umbilical cord blood stem cells. Best Pract Res Clin Obstet Gynaecol. 2004 Dec;18(6):893-908

33. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-masiuk M. Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia, 2007; 21: 297-303.
34. Weiss ML, Troyer DL. Stem cells in the umbilical cord. Stem Cell Rev. 2006; 2(2):155-62
35. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World J Stem Cells. 2014 Apr 26; 6(2):195-202.
36. Liu, G, Ye, X, Zhu, Y, Li, Y, Sun, J, Cui, L, et al. Osteogenic differentiation of GFP-labeled human umbilical cord blood derived mesenchymal stem cells after cryopreservation. Cryobiology, 2011; 63: 125-128
37. Wang H, Qiu X, Ni P, et al. Immunological characteristics of human umbilical cord mesenchymal stem cells and the therapeutic effects of their transplantion on hyperglycemia in diabetic rats. *Int J Mol Med*. 2014;33(2):263-270.
38. Mennan C, Wright K, Bhattacharjee A, Balain B, Richardson J, Roberts S. Isolation and characterisation of mesenchymal stem cells from different regions of the human umbilical cord. Biomed. Res. Intern. 2013: 916136.
39. Wang Z. G, He Z. Y, Liang S, Yang Q, Cheng P, Chen A. M. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res. Ther. 2020; 11:511
40. Filip A, Dabrowski AB, Kulesza A, Sladowska A, Zolocinska A, Gala K, et al. Comparison of the paracrine activity of mesenchymal stem cells derived from human umbilical cord, amniotic membrane and adipose tissue. J Obstet Gynaecol Res. 2017;43(11):1758–68.
41. Yao J, Zheng J, Cai J, Zeng K, Zhou C, JiebinZhang SL, et al. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response. FASEB J. 2019;33(2):1695–710.
42. Ruan Z-B, Chen G-C, Zhang R, Zhu L. Circular RNA expression profiles during the differentiation of human umbilical cord–derived mesenchymal stem cells into cardiomyocyte-like cells. J Cell Physiol. 2019; 234(9):16412–23.
43. Lian J, Lv S, Liu C, Yang L, Wang S, Guo X, et al. Effects of serial passage on the characteristics and cardiac and neural differentiation of human umbilical cord Wharton's jelly-derived mesenchymal stem cells. Stem Cells Int. 2016; 9291013.
44. Zhang Y, Li Y, Li W, Cai J, Yue M, Jiang L, et al. Therapeutic effect of human umbilical cord mesenchymal stem cells at various passages on acute liver failure in rats. Stem Cells Int. 2018; 7159465.
45. Xie, Q, Liu, R, Jiang, J. Peng J, Yang C, Zhang W, et al. What is the impact of human umbilical cord mesenchymal stem cell transplantation on clinical treatment?. Stem Cell Res Ther 11, 519 (2020)

46. Hofmann J, Hackl V, Esser H, Meszaros AT, Fodor M, Ofner D, Troppmair J, Schneeberger S, Hautz T. Cell-based regeneration and treatment of liver diseases. Int J Mol Sci. 2021;22:10276.
47. Li GC, Ye QH, Xue YH, Sun HJ, Zhou HJ, Ren N, et al. Human mesenchymal stem cells inhibit metastasis of a hepatocellular carcinoma model using the MHCC97-H cell line. Cancer Sci. 101:2546–2553. 2010. View Article : Google Scholar : PubMed/NCBI
48. Yun JW, Ahn JH, Kwon E, et al. Human umbilical cord-derived mesenchymal stem cells in acute liver injury: Hepatoprotective efficacy, subchronic toxicity, tumorigenicity, and biodistribution. *Regul Toxicol Pharmacol*. 2016;81:437-447.
49. Li Q, Pang Y, Liu T, Tang Y, Xie J, Zhang B, et al. Effects of human umbilical cord-derived mesenchymal stem cells on hematologic malignancies. Oncol Lett. 2018; 15(5):6982–90.
50. Zong C, Zhang H, Yang X. The distinct roles of mesenchymal stem cells in the initial and progressive stage of hepatocarcinoma. Cell Death Dis. 2018;9:345
51. Liu J, Shi Y, Han J, Zhang Y, Cao Z, Cheng J. Quantitative tracking tumor suppression efficiency of human umbilical cord-derived mesenchymal stem cells by bioluminescence imaging in mice hepatoma model. Int J Stem Cells. 2020; 13:104-115
52. Jiang W, Tan Y, Cai M. Human umbilical cord MSC-derived exosomes suppress the development of CCl(4)-induced liver injury through antioxidant effect. Stem Cells Int. 2018; 6079642.
53. Tang Y-M, Bao W-M, Yang J-H. Umbilical cord-derived mesenchymal stem cells inhibit growth and promote apoptosis of HepG2 cells. Mol Med Rep. 2016; 14: 2717-2724.
54. Endaya B, Guan SP, Newman JP. Human mesenchymal stem cells preferentially migrate toward highly oncogenic human hepatocellular carcinoma cells with activated EpCAM signaling. Oncotarget. 2017; 8: 54629-54639
55. Chaput B, Foucras L, Le Guellec S, Grolleau JL, Garrido I. Recurrence of an invasive ductal breast carcinoma 4 months after autologous fat grafting. Plast Reconstr Surg. 2013; 131: 123e-124e.
56. Shen C, Lie P, Miao T. Conditioned medium from umbilical cord mesenchymal stem cells induces migration and angiogenesis. Mol Med Rep. 2015; 12(1): 20–30