ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
March 2022 • Vol.25

Effect of Mesenchymal Stem Cell-Derived Exosomes on Head & Neck Squamous Cell Carcinoma Cell Line in-Vitro Study

Amal Shehab Eldin Shafiaa[1], Houry Moustafa Baghdadi[2], Dina Sabry[3], Rehab Fouad[4]

Background: Oral cancer causes approximately 7,800 annual deaths and has an average range of 5-year mortality rate between 53% and 56%. Mesenchymal stem cells (MSCs) are multipotent cells that differentiate into several cell types. Due to the fact that they can be recruited at sites of inflammation and tissue repair, the role of MSCs in regenerative medicine and their potential use as tools for gene delivery have been extensively studied. Mesenchymal stem cell-derived exosomes (MSC-derived exosomes) are expected to be a potential treatment method for squamous cell carcinoma of the head and neck (HNSCC). MSC-derived exosomes might play a significant role in the tumor microenvironment, particularly affecting tumor vasculature, metastatic potential and progression.
Aim: To determine the efficacy of exosomes derived from MSCs on HNSCC cell line in respect to metastasis by evaluating the expression of CRKI.
Material and Methods: Laryngeal carcinoma cell line (HEp-2) was cultured for 24 and 48 hours with two different doses of exosomes (10ng/ml and 20ng/ml) obtained from adipose derived MSCs. Western blot and electron microscopy were used to characterize and image the exosomes. To act as a control, a set of cells was left untreated. The potential anti-metastatic effect of the exosomal cargo were investigated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) through the measurement of (CRKI) gene expression levels in cultured HEp-2 cells following exosome absorption.
Results: MSC-derived exosomes reduced the viability of HEp-2 cells in a dosage and time-dependent manner. In treated HEp-2 cells, exosomes drastically reduced CRKI gene expression.
Conclusion: Based on the current findings, MSC-derived exosomes may be a viable supplementary anticancer therapy for HNSCC by reducing these cells' invasiveness.

Keywords: Exosomes; MSC; CRKI; metastasis; invasion; HEp-2

1. Assistant Lecturer, Oral Pathology Department, Faculty of Dentistry, Egyptian Russian University, Cairo, Egypt.
2. Professor of Oral Pathology, Head of Oral Pathology Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
3. Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University.
4. Lecturer of Oral Pathology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.
Corresponding author: Amal Shehab Eldin Shafiaa, email: shehabamal3@gmail.com

Introduction

Head and neck cancer (HNC), the sixth most frequent cancer globally, is a horrible illness that claims the lives of up to 300,000 people each year. The five-year survival rate has lately been estimated to be between 40-60% due to the intricacy of most head and neck anatomical locations, resulting in late identification and challenging surgical operability (Gorphe, 2019).

Mesenchymal stem cells (MSCs) are multipotent cells that may develop into osteoblasts, chondrocytes, adipocytes, and other mesodermal lineages. Despite widespread interest in the role of MSCs in cancer development, the link between MSCs and tumor cells remains unknown. Several studies have shown that MSCs contribute to tumor progression and metastasis, whereas others have found that MSCs inhibit tumor growth. An increasing amount of data suggests that MSCs modulate the tumor microenvironment mostly through paracrine activity and the production of numerous trophic substances (Djouad et al., 2003).

Exosomes are small (30 to 100 nm) membrane-bound particles released by most cells. They are thought to play important roles in intercellular communication by transferring genetic molecules such as coding and non-coding RNAs (mRNAs and miRNAs) to modulate cellular activities in recipient cells (Ståhl et al., 2019).

The effect of MSC-derived exosomes on tumor growth has received a lot of attention in the last decade. Exosomes generated from MSCs influence tumor formation in both supportive and suppressive ways. Among the potential pathways, the miRNA content of exosomes has received a lot of attention. MSC-derived exosomes may be important in the tumor microenvironment, particularly in tumor vasculature (Pakravan et al., 2017).

The CT10 chicken retrovirus oncogene fusion product, v-CRK (Mayer et al., 1988), was the source of the initial isolation of CRK gene. Cellular homologues of v-CRK include the c-CRK gene, which encodes two alternatively spliced mRNAs that give rise to two proteins (c-CRKI and c-CRKII), and a second gene, c-CRKL. CRKI proteins are composed of one Src homology 2 (SH2) and, one or two Src homology 3 (SH3) domains. They are adaptor proteins that direct the assembly of multiprotein signaling complexes (Matsuda et al., 1992).

CRK's function in mammalian cells is determined through investigations on its overexpression. Although CRKII (Imaizumi et al., 1999) and CRKL (Guris et al., 2001) knockout mice have been established and RNA interference of CrkII has been shown to reduce membrane ruffling of aortic endothelial cells (Nagashima et al., 2002), only little analysis of cells derived from these mice has been published to date. Rodrigues et al., (2005) looked at the correlation between CRK protein levels in human breast cancer and employed RNA interference directed against CRKI/II in human cancer cell lines to look into a necessity for CRK in migration and invasion in human cancers. The researchers demonstrated increased CRKI/II protein levels in breast adenocarcinoma and demonstrated a critical function for CRK adaptor proteins in the improved migration and invasion signaling pathways of several human cancer cell lines.

Material and Methods

The Research Ethics Committee of the Faculty of Dentistry at Ain Shams University gave its approval to each technique before it was carried out in the Medical Biochemistry and Molecular Biology Department (Tissue Culture Unit) of Cairo University's Faculty of Medicine in Egypt.

1- Isolation and Culturing of Adipose-Derived Mesenchymal Stem Cells (ADSCs)

A sample of adipose tissue, obtained from lipoaspirates harvested through a liposuction procedure in a plastic surgery clinic, was chopped and digested with collagenase type I solution. Following digestion, the sample was successively diluted, filtered, and centrifuged at 600 x g for 7 minutes to produce a pallet from which MSCs were extracted and plated for sub-culturing in order to be examined under an inverted light microscope.

2. Characterization and Assessment of Stemness

Mesenchymal cell markers (CD29, CD44, CD73, CD90, and CD105), hematopoietic markers (CD34 and CD45), endothelial cell markers (CD34 and CD31), and other stem cell markers were examined for expression in the isolated ADSCs (CD117). To create a homogeneous population that exclusively expresses mesenchymal cell markers, the stem cell endothelial or hematopoietic markers were gradually eliminated.

3- Isolation and Characterization of ADSC-Derived Exosomes

After first ultra-centrifuging the culture medium, aspirating the supernatant, and then pelleting the exosomes, the exosomes were finally re-dissolved in PBS. Using the Bradford protein assay (kit from Bio-Rad Laboratories Inc., USA), the protein content of the exosomes was assessed in order to calculate the exosome yields or exosome dosage for following research. For this investigation, two exosomal concentrations (10 and 20 ng/ml) were used. The presence of exosomal proteins CD31 and CD81 was confirmed in the isolated exosomes using the Western Blot method. The existence of cup-shaped vesicles with a diameter ranging from 10 to 1500 nm was discovered using a transmission electron microscope to observe the exosomes' size and form.

4- Culturing of the Cancer Cell Line

The HEp-2(ATCC® CCL-23TM) human laryngeal squamous cell carcinoma cell line was graciously provided by the Cell Culture Department-VACSERA, Egypt for use in this study. In addition to antibiotics (100 units/ml penicillin and 100 g/ml streptomycin), 10% fetal bovine serum (FBS) (HyClone), 2 mM glutamine, and sodium bicarbonate were added to Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen/Life Technologies) to culture HEp-2 cells. In a humidified incubator with 95% air and 5% CO2, the cultivated cells were incubated at 37°C. The pH was then maintained at 7.4 while HEp-2 cells were processed via a Trypsin-EDTA solution (0.25%) and phosphate-buffered saline (PBS) solution (both from Sigma-Aldrich, USA).
Control HEp-2 cells were grown for 24 hours (CT1) and 48 hours without any treatments (CT2).

According to the length and exosomal concentration, the exosome-treated HEp-2 cells were divided into four categories. The experimental groups employed in this investigation are listed in Table 1.

Table (1): Cell culture experimental groups

Group	Culture description
CT1	Control group (untreated HEp-2 cells) in 24 hours
CT2	Control group (untreated HEp-2 cells) in 48 hours
Exo1T1	HEp-2 cell line treated with exosomes for 24 hours (10 ng/ml)
Exo1T2	HEp-2 cell line treated with exosomes for 48 hours (10 ng/ml)
Exo2T1	HEp-2 cell line treated with exosomes for 24 hours (20 ng/ml)
Exo2T2	HEp-2 cell line treated with exosomes for 48 hours (20 ng/ml)

5- Microscopic Analysis

Photomicrographs of HEp-2 cells were taken before and after treatment with MSC-derived exosomes at 24 and 48 hours in 10 and 20 ng/ml concentrations for microscopic analysis using an inverted light microscope (original magnification x100 + oil).

6- MTT Viability Assay

Sigma-Aldrich in the USA was used to acquire the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) viability kit. This assay, which measures the viable fraction of cultured cells based on their metabolic activity, does so primarily by reducing the yellow tetrazolium salt in MTT solution into purple insoluble formazan crystals, which can then be solubilized and measured by spectrophotometric methods. Viable cells retain an active mitochondrial oxidoreductase enzyme that does this. The cells were then treated for 4 hours with 0.5 mg/ml MTT reagent after being incubated for 24 and 48 hours. When the purple dye was clearly visible after that, the MTT solubilization solution was added. As the optical density (OD) was measured using an ELISA microplate reader (ROBONIK P2000 EIA reader, India) at a wavelength range of 490-630 nm, absorbance values in each well were calculated. For every exosomal concentration and time period, the experiment was run three times. Additionally, triple measurements of the absorbance of untreated control HEp-2 cells were made at each time interval. The absorbance mean of each subgroup was determined and utilized in the equation below to get the viability percentage:

Viability % = (absorbance mean of treated cells/ absorbance mean of control cells) X 100.

7- Assessment of CRKI Gene Expression using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The RNA was extracted, purified and reverse transcribed into complementary DNA (cDNA) using the reverse transcriptase RT-PCR kit. The primer sequences of the target CRKI gene and the reference housekeeping gene (Beta-actin) were (F: TAGGCAAGTCACATGCATTG; R:AAGGAAGACCCTTTCCACGT) and (F: GGC GGCACCACCATGTACCCT, R: AGG GGCCGGACTCGTCATACT), respectively. Then thermal cycling was run and the relative quantification (RQ) of CRKI gene expression was calculated according to (delta-delta Ct) (ΔΔCt). The RQ of the target gene was calculated by taking 2-ΔΔCt as follows:

ΔΔCt = [(Ct target, Sample)-(Ct ref, Sample)]-[(Ct target, Control)-(Ct ref, Control)]

Where: Ct target, Control = Ct value of gene of interest in control DNA

Ct ref, Control = Ct value of reference gene in control DNA

Ct target, Sample = Ct value of gene of interest in tested sample

Ct ref, Sample = Ct value of reference gene in tested sample

8- Statistical Analysis

The statistical software for the social sciences (SPSS) version 26 was used to code and input the data (IBM Corp., Armonk, NY, USA). The mean and standard deviation were used to summarize the data. Analysis of variance (ANOVA) was used to compare the differences between the groups, and the Tukey post hoc test was used to compare the differences between each pair of groups. P-values of 0.05 or less were regarded as statistically significant.

Results

Microscopic Examination Results

Figure 1 provides an overview of the microscopic results of evaluation of all groups by analyzing the digital photomicrographs of HEp-2 cells.

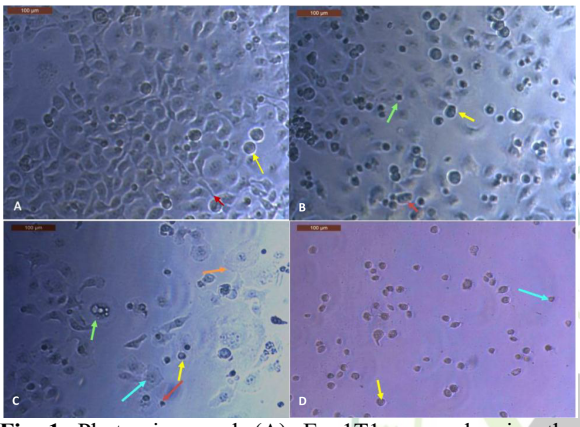

Fig. 1: Photomicrograph (A): Exo1T1 group showing the rounding up of some degenerating cancer cells (yellow arrow) while the viable cells retained the large polyhedral uniform outline (red arrow); (B): Exo2T1group showing the cytoplasmic shrinkage (yellow arrow), loss of regular outline (red arrow) and apoptotic body (green arrow). (C): Exo1T2 group showing shrunken rounded up apoptotic cell (yellow arrow) and apoptotic body (red arrow). The orange arrow points to a swollen necrotic cell. The green arrow points to cytoplasmic vacuolization characteristic of necrosis. Note the disintegrating nucleus (karyolysis) of necrotic cells (light blue arrow). (D): Exo2T2 group showing remarkable generalized decrease in cell size (yellow arrow) with brisk formation of apoptotic bodies (light blue), distinguishing apoptosis as the main type of cell death here (original magnification x100 + oil).

MTT Assay Results

The recorded viability percentage data of all experimental groups are shown in table (2) and figure (2).

Table (2): Mean ± SD of viability percentage of all experimental groups.

	CT1	Exo1T1	Exo2T1	CT2	Exo1T2	Exo2T2	P-value
Viability %	1.82^{cd}±0.62	1.23 ^{bc}±0.47	0.72^{ab}±0.33	2.28^{d}±0.81	0.79^{abc}±0.20	0.34^{ae}±0.16	0.000*

*Significance is at *p*-value < 0.05
Different superscripts mean that the statistical difference between groups is significant.

The mean viability % varied significantly across all groups, according to the ANOVA test (p-value = 0.000). The decrease in viability % following exosomal therapy at the lower dose (10 ng/ml) for 24 hours, compared to the control group (CT1), was minimal and inconsequential (p-value=0.097), according to a post-hoc multiple-comparison test. In comparison to the control group, there was a statistically significant decline in mean viability % throughout the treatment with the increased exosomal concentration (20ng/ml) for 24 hours (CT1). There was a statistically significant difference in the mean viability % for the 48-hour period between CT2 and both Exo1T2 and Exo2T2 (p 0.001). It's interesting to note that for both periods, the drop in mean viability % from higher to lower exosomal concentrations was statistically non-significant (p-values of 0.203 and 0.319, respectively).

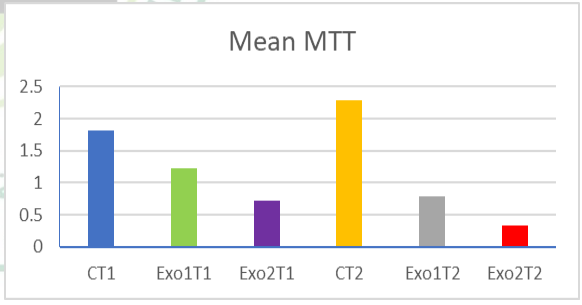

Fig. 2: Bar chart showing the mean viability percentages of HEp-2 cells for the experimental groups.

Quantitative-Real Time Polymerase Chain Reaction (qRT-PCR) Results:

The mean values of CRKI gene expression in all groups are demonstrated in table (3) and figure (3).

Table (3): Mean ± SD of CRKI gene expression in all experimental groups.

	CT1	Exo1T1	Exo1T2	CT2	Exo2T1	Exo2T2	P-value
CRKI gene expression	2.93^{c}±0.85	1.89^{b}±0.71	1.06^{ab}±0.51	3.85^{d}±1.06	1.30^{ab}±0.26	0.67^{a}±0.24	0.000*

*Significance is at *p*-value < 0.05
Different superscripts mean that the statistical difference between groups is significant.

ANOVA analysis revealed a statistically significant difference between Exo1T1 and CT1 in the mean value of CRKI gene expression during a 24-hour period (p-value = 0.013). Exo2T1 and CT1's mean CRKI gene expression levels differed statistically significantly from one another (p-value 0.001). After 48 hours, a post-hoc multiple-comparison test comparing CT2 and both Exo1T2 and Exo2T2 revealed a highly statistically significant drop in mean CRKI gene expression (p-value 0.001).

The mean values of CRKI gene expression did not show a statistically significant difference between the two exosomal concentrations at any of the two time points (p-values = 0.086 and 0.316, respectively).

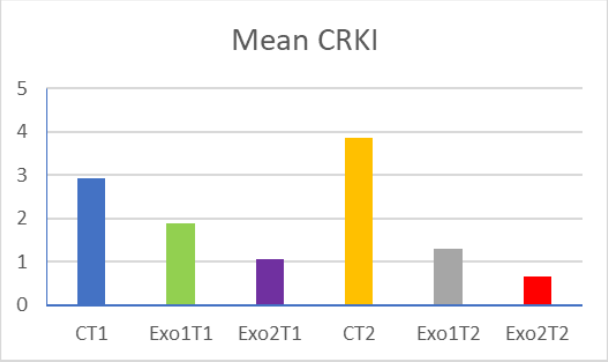

Fig. 3: Bar chart showing the mean values of CRKI gene expression in HEp-2 cells for the experimental groups

Discussion

Surgical treatment to remove HNSCC is usually of high risk, life threatening and may cause deformation. Cancer progression is controlled by the capacity of cancer cells to maintain angiogenesis. Angiogenesis is necessary for solid tumors like HNSCC to begin the process of invasion and metastasis because it makes sure the cancer cells have enough oxygen, nutrients, and time to excrete metabolites. Additionally, the cancer cells are capable to access the main circulation to achieve distant metastasis by their ability to produce leaky vasculature (Majidpoor and Mortezaee, 2021).

The aim of this study was to investigate the effect of exosomes derived from MSCs on HNSCC cell line in respect to metastasis by evaluating CRKI gene expression.

Emerging research on the anti-tumorigenic properties of exosomes generated from MSCs showed that these cells can suppress the expression of genes linked to angiogenesis, self-renewal and cell cycle progression. This can stop the spread and growth of cancer (Zhou et al., 2018; Karaoz et al., 2019). On the other hand, several studies have shown that MSCs can encourage oncogenesis through their paracrine action. Proliferation, angiogenesis and drug resistance-related signaling pathways are altered by the generated protumorigenic chemicals and miRNAs. These elements can also inhibit the production of genes that promote apoptosis and tumor suppression. This is how the MSC-derived secretome can alter the phenotypes and behavior of cancer (Qi et al., 2017; Zhao et al., 2019).

In comparison to healthy control, it was discovered that cancer patients had more exosomes identified in their cells. This research demonstrated the critical role exosomes play in the initiation and spread of a variety of cancers (Suchorska and Lach, 2016).

Paracrine factors in MSC-derived exosomes can transmit their contents to nearby tumor cells or cause phenotypic changes in recipient cells, which may affect tumor growth both in vitro and in vivo (Camussi et al., 2010).

Numerous researchers have been interested in the MSC exosome area as a result of its recent promising results. MSC-derived exosomes have a tremendous potential for therapeutic uses given how simple it is to separate and manipulate their surface and contents. Exosomes lipid and

protein makeup, which improves exosomal stability and slows circulatory clearance, is another characteristic that makes these vesicles ideal transporters. These vehicles could be viewed as the next-generation drug delivery system because of their tiny size, lack of toxicity, target specificity, and ability to be tolerated by the body. Furthermore, compared to MSCs, exosomes might be produced on a large scale, are simpler to handle, cost less, and do not potentially present ethical or legal issues. These properties show exosomes potential for prospective medicinal uses (Vakhshiteh et al., 2019).

The gene that produces the protein CRKI is overexpressed in many forms of human cancer and is associated with bad prognosis. The proliferation, transformation, migration, invasion, epithelial-to-mesenchymal transition, resistance to chemotherapeutic medicines, in vivo tumor development and metastasis of tumor cells are all influenced by the level of expression of CRKI in different tumor cell lines (Zhou et al., 2018).

This work employed the Western Blot approach to characterize exosomes by identifying the proteins that are abundant in exosomes (exosomal markers). It is an extremely sensitive technique for identifying certain proteins in an intricate antigenic mixture (Burnette, 1981). Because the proteins are initially separated by electrophoresis according to their molecular weight to prevent non-specific staining, the Western Blot is more accurate than other protein detection techniques like ELISA and immunocytochemistry (Mahmood and Yang, 2012). Furthermore, because ELISA only detects proteins in liquid form, it can only identify cytoplasmic and secreted proteins, whereas Western Blot can identify nuclear, cytoplasmic, and cell membrane proteins (Mahmood and Yang, 2012; Gan and Patel, 2013). The RNA and protein levels of gene expression may also be found and tracked using Western Blot (Bran et al., 2009).

By examining the mesenchymal markers (CD29, CD44, CD73, CD90, and CD105) and omitting other types of stem cells that express endothelial or hematological markers, the current study's goal of characterizing ADSCs and evaluating the MSC phenotype was attained. This was necessary to make sure that the yield of exosomes collected was solely from the MSC lineage, which is renowned for its ability to modulate tumor growth.

The use of ADSCs in our current research was justified by their greater applicability than BMSCs since it is possible to collect stem cells from adipose tissue through frequent surgical procedures like liposuction. Additionally, ADSCs are more adept at self-renewal and trans-differentiation into a wide range of cell lineages (Lambert et al., 2009).

In the current investigation, treated and untreated HEp-2 cells were cultured for 24 and 48 hours at 10 and 20 ng/ml doses, respectively. There was a statistically significant difference in the mean viability % between Exo1T1 and Exo2T2 (p-value = 0.203). This supports the idea that, as demonstrated by Karaoz et al. (2019), the concentration and length of treatment with MSC-derived exosomes on HEp-2 cells play an important role in reducing cancer cell growth.

Treatment with MSC-derived exosomes significantly reduced the mean viability percentage of HEp-2 cells at both time points (24 and 48 hours), demonstrating the effectiveness of the treatment in reducing the proliferation of cancer cells even though the used concentrations were statistically insignificant when compared to one another.

Exosomes released by menstrual mesenchymal stem cells (MenSCs) have been discovered as inhibitors of tumor angiogenesis and modulators of the tumor

cell secretome in prostate and breast cancer, according to Rosenberger's et al. (2019) findings. Exosome treatment of cultivated endothelial cells enhanced cytotoxicity and, in a dose-dependent way, decreased VEGF release and angiogenesis in their experiment. Additionally, they showed a significant anticancer effect upon intra-tumoral injection of exosomes at different durations (4, 8 and 16 hours) using hamster buccal pouch carcinoma as a preclinical model for human OSCC, as the mean viability percentages of cancer cells decreased with increasing the durations upon treatment by MenSC exosomes.

In contrast, Gu et al. (2016) found that the treatment group treated with MSC-derived exosomes had an 8-fold rise in the number of gastric cancer (HGC-27) cells, which was attributed to the activation of the protein kinase B (Akt) signaling pathway.

A cytological evaluation of cultivated HEp-2 cells was performed in this study using an inverted light microscope to evaluate the effect of MSC-derived exosomes on the morphology of the cultured cancer cells. This process was not only incredibly educational and economical, but it also had the advantages of simplicity and ease.

Correlating the molecular results from the qRT-PCR and MTT viability tests with the anticipated effects of exosomal therapy as shown on the cytomorphological level was crucial. In contrast to the untreated control cells, the exosome-treated HEp-2 cells clearly displayed cytological abnormalities that indicated a decline in cell viability, degeneration, and even overt symptoms of cell death, such as apoptosis and necrosis.

The morphological changes observed in HEp-2 cells after exosome treatment at two different concentrations and durations included a decrease in cellular density, cytoplasmic shrinkage, loss of regular cellular and nuclear contour, as well as obvious signs of cell degeneration indicative of apoptotic cell death. On the other hand, in addition to karyolysis, cytoplasmic swelling and vacuolization were morphological signs that suggested necrotic cell death. Intriguingly, this cytological morphology was noticed in a dose/time-dependent way and was in contrast to the untreated control HEp-2 cells, which still had their characteristically large, elongated shape.

To measure the level of CRKI gene expression in treated and untreated HEp-2 cells, qRT-PCR was the method of choice in the current investigation. PCR is generally a superiorly sensitive test that allows efficient amplification of specific DNA sequences to generate millions of copies for the gene of interest. qT-PCR facilitates a quantitative measurement of the gene under investigation as the amplification reaction proceeds.

Treatment with MSC-derived exosomes significantly reduced CRKI gene expression at both time points (24 and 48 hours), demonstrating the treatment's efficacy in lowering HEp-2 cell invasiveness even though the employed doses (10 and 20 ng/ml) were statistically insignificant compared to one another.

The findings of Guo et al. (2018), who investigated hepatocellular carcinoma HepG2 cells, are consistent with our findings. They discovered that exosomal miR-429 directly targets CRKI by altering the Raf/MEK/ERK-EMT pathway, hence disrupting the ensuing signal networks of migration and invasion.

Different factors may be responsible for the various characteristics of MSCs-derived exosomes as well as the variations in their effects on the phenotype and behavior of cancer. These factors include the origin of the MSCs, the experiment design, the growth conditions for the stem cells, the various therapies given to those cells, the administration techniques employed, as well as the cancer environment and interactions

with the stem cells (Yassine and Alaaeddine, 2022).

However, the encouraging findings of our study require additional confirmation and validation by vigorous future work in the field of natural nanoparticle bioengineering, eliminating the use of chemotherapeutic medicines with their severe associated side effects.

Conclusion

According to the current research, MSC-derived exosomes have the potential to be an additional anticancer therapy for HNSCC by reducing the ability of these cells to invade.

References

Bran G., Goessler U., Hormann K., Riedel F. and Sadick H.: Keloids: current concepts of pathogenesis. International Journal of Molecular Medicine 2009; 24: 283-293.

Burnette W.: Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Analytical Biochemistry 1981; 112: 195-203.

Camussi G., Deregibus M., Bruno S., Cantaluppi V. and Biancone L.: Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney International 2010; 78: 838-848.

Djouad F., Plence P., Bony C., Tropel P. and Apparailly F.: Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 2003; 102: 3837-3844.

Gan S. and Patel K.: Enzyme immunoassay and enzyme-linked immunosorbent assay. Journal of Investigative Dermatology 2013; 133: 12-14.

Gorphe P.: A comprehensive review of HEp-2 cell line in translational research for laryngeal cancer. American Journal of Cancer Research 2019; 9: 644-649.

Gu Z., Shi S., Zhang Q., Xia Y., You B., Shan Y., Bao L., Li L. and You Y.: Mesenchymal stem cell-derived exosomes facilitate nasopharyngeal carcinoma progression. American Journal of Cancer Research. 2016; 6: 459-463.

Guo L., Fang T., Lv H., Lv G., Li T., Wang C., Han Q., Yu L., Su B., Huang S. and Cao D.: Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nature Communications. 2018; 9: 1-3.

Guris D., Fantes J., Tara D., Druker B. and Imamoto A.: Mice lacking the homologue of the human 22q11.2 gene CRKL phenocopy neurocristopathies of DiGeorge syndrome. Nature Genetics 2001; 27: 293-298.

Imaizumi T., Araki K. and Miura K.: Mutant mice lacking Crk-II caused by the gene trap insertional mutagenesis: Crk-II is not essential for embryonic development. Biochemical and Biophysical Research Communication 1999; 266: 569-574.

Karaoz E., Sun E. and Demir C.: Mesenchymal stem cell-derived exosomes do not promote the proliferation of cancer cells in vitro. International Journal of Physiology, Pathophysiology and Pharmacology 2019; 11: 177-189.

Lambert A., Zandonai A., Bonatto D., Machado D. and Henriques J.: Differentiation of human adipose-derived adult stem cells into neuronal tissue: does it work?. Differentiation 2009; 77:221-228.

Mahmood T. and Yang P.: Western blot: technique, theory and trouble shooting. North American Journal of Medical Sciences 2012; 4: 429-435.

Majidpoor J. and Mortezaee K.: Angiogenesis as a hallmark of solid tumors-clinical perspectives. Cellular Oncology 2021; 44: 715-737.

Matsuda M., Reichman C. and Hanafusa H.: Biological and biochemical activity of v-Crk chimeras containing the SH2/SH3 regions of phosphatidylinositol-specific phospholipase C-γ and Src. Journal of Virology 1992; 66: 115-121.

Mayer B., Hamaguchi M. and Hanafusa H.: A novel viral oncogene with structural similarity to phospholipase C. Nature 1988; 332: 272-275.

Nagashima K., Endo A. and Ogita H.: Adaptor protein Crk is required for ephrin-B1-induced membrane ruffling and focal complex assembly of human aortic endothelial cells. Molecular Biology of the Cell 2002; 13: 4231-4242.

Pakravan K., Babashah S., Sadeghizadeh M., Mowla S., Mossahebi-Mohammadi M., Ataei F., Dana N. and Javan M.: MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the

mTOR/HIF-1α/VEGF signaling axis in breast cancer cells. Cellular Oncology 2017; 40: 457-462.

Qi J., Zhou Y., Jiao Z., Wang X., Zhao Y., Li Y., Chen H., Yang L., Zhu H. and Li Y.: Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth through hedgehog signaling pathway. cellular physiology and biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry and Pharmacology 2017; 42: 2242-2254.

Rodrigues S., Fathers K., Chan G., Zuo D., Halwani F., Meterissian S. and Park M.: CrkI and CrkII function as key signaling integrators for migration and invasion of cancer cells. Molecular Cancer Research 2005; 3: 183-194.

Rosenberger L., Ezquer M., Lillo-Vera F., Pedraza P., Ortúzar M., González P., Figueroa-Valdés A., Cuenca J., Ezquer F., Khoury M. and Alcayaga-Miranda F.: Stem cell exosomes inhibit angiogenesis and tumor growth of oral squamous cell carcinoma. Scientific Reports 2019; 9: 1-2.

Ståhl A., Johansson K., Mossberg M., Kahn R. and Karpman D.: Exosomes and microvesicles in normal physiology, pathophysiology and renal diseases. Pediatric Nephrology 2019; 34: 11-20.

Suchorska W. and Lach M.: The role of exosomes in tumor progression and metastasis (Review). Oncololgy Reports 2016; 35: 1237-1244.

Vakhshiteh F., Atyabi F. and Ostad S.: Mesenchymal stem cell exosomes: a two-edged sword in cancer therapy. International Journal of Nanomedicine 2019; 14: 2847-2852.

Yassine S. and Alaaeddine N.: Mesenchymal stem cell exosomes and cancer: controversies and prospects. Advanced Biology 2022; 6: 2101-2115.

Zhao T., Sun F., Liu J., Ding T., She J., Mao F., Xu W., Qian H. and Yan Y.: Emerging role of mesenchymal stem cell-derived exosomes in regenerative medicine. Current Stem Cell Research and Therapy 2019; 14: 482-494.

Zhou J., Tan X., Tan Y., Li Q., Ma J. and Wang G.: Mesenchymal stem cell derived exosomes in cancer progression, metastasis and drug delivery: a comprehensive review. Journal of Cancer 2018; 9: 3129-3135.