Research Article

The role of stem cell markers in choriocarcinoma: Immunocytochemical analysis in JAR cell line

Koryokarsinomda kök hücre belirteçlerinin rolü: JAR hücre hattında immünositokimyasal analiz

Ertan KATIRCI[1], Remziye KENDİRCİ[2], H.Seda VATANSEVER[3,4]

[1] Ahi Evran University Faculty of Medicine, Kırşehir,Türkiye
[2] Akdeniz University Faculty of Medicine, Antalya, Türkiye
[3] Faculty of Medicine, Department of Histology and Embryology, Manisa Celal Bayar University, Manisa, Türkiye
[4] DESAM Institute, Near East University, Lefkoşa (Nicosia), 99138, North Cyprus via Mersin 10, Türkiye

ABSTRACT

Objective: Choriocarcinoma is a type of cancer that originates from placental trophoblastic tissue and has a high malignant potential. The aim of this study was to investigate the distribution of stem cell and cancer stem cell markers in the JAR cell line. The study is to provide information about the biology of choriocarcinoma and the mechanisms of resistance to treatment.

Materials and Methods: JAR cells were cultured in RPMI 1640 culture medium. Colony formation assay was performed in JAR cells. The expression of OCT3/4, CD133, C-KIT, SALL4 and CD90 markers were analysed using indirect immunoperoxidase method. Healthy endometrial epithelial cell line RL95-2 was used for statistical analysis.

Results: JAR cells showed statistically high colony formation efficiency compared to the RL95-2 group. Immunocytochemical analyses showed strong immunoreactivity for CD133 and moderate immunoreactivity for SALL4. C-KIT, OCT3/4 and CD90 showed weak immunoreactivity.

Conclusion: The JAR cell line has emerged as a valuable model for trophoblastic cell invasion and placentation studies in choriocarcinoma. Markers such as CD133 and SALL4 provide important information about stem cell-like characteristics and choriocarcinoma malignancy. Lower expressions of C-KIT, OCT3/4 and CD90 reflect heterogeneous stem cell profiles in JAR cells. This diversity provides important insights into the complex nature of choriocarcinoma stem cells and sheds light on potential therapeutic approaches.

Keywords: Choriocarcinoma, JAR cell line, stem cell markers, cancer stem cells, trophoblastic malignancy.

ÖZ

Amaç: Koryokarsinom, plasental trofoblastik dokudan köken alan ve yüksek malignite potansiyeline sahip bir kanser türüdür. Bu çalışmanın amacı, JAR hücre hattında kök hücre ve kanser kök hücre belirteçlerinin dağılımını incelemek ve koryokarsinom biyolojisi ile tedaviye direnç mekanizmalarına dair bilgiler sağlamaktır.

H.Seda VATANSEVER
Faculty of Medicine, Department of Histology and Embryology, Manisa Celal Bayar University, Manisa, Türkiye
DESAM Institute, Near East University, Lefkoşa (Nicosia), 99138, North Cyprus via Mersin 10, Türkiye
E-posta: hsedavatansever@gmail.com
https://orcid.org/ 0000-0002-7415-9618

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Gereç ve Yöntem: JAR hücreleri, RPMI 1640 kültür ortamında kültüre edildi. Indirekt immünoperoksidaz yöntemi kullanılarak OCT3/4, CD133, C-KIT, SALL4 ve CD90 belirteçlerinin ifadesi analiz edildi.

Bulgular: JAR hücre hattı, 2D kültür ortamında sferoid benzeri yapılar oluşturmuştur. İmmünositokimyasal analizler, CD133 için güçlü immünoreaktivite ve SALL4 için hafif immünoreaktivite göstermiştir. CD90 negatif gözlenmiştir, C-KIT ve OCT3/4 ise zayıf immünoreaktivite sergilemiştir.

Sonuç: JAR hücre hattı, koryokarsinomda trofoblastik hücre invazyonu ve plasentasyon araştırmalarında değerli bir model olarak öne çıkmaktadır. CD133 ve SALL4 gibi belirteçler, kök hücre benzeri özellikler ve koryokarsinom malignitesi ile ilgili önemli bilgiler sağlamaktadır. C-KIT ve OCT3/4'ün daha düşük ekspresyonları, JAR hücrelerinde heterojen kök hücre profillerini yansıtmaktadır. Bu çeşitlilik, koryokarsinom kök hücrelerinin karmaşık yapısına dair önemli bilgiler sağlayarak, potansiyel tedavi yaklaşımlarına ışık tutmaktadır.

Anahtar Kelimeler: Koryokarsinom, JAR hücre hattı, kök hücre belirteçleri, trofoblastik malignite, kanser kök hücreleri.

INTRODUCTION

Choriocarcinoma is a highly aggressive malignancy arising from placental trophoblastic tissue, characterized by rapid growth, early metastasis, and high mortality rates. Although choriocarcinoma is known to be sensitive to chemotherapy compared to other trophoblastic diseases, resistance to treatment can occur in advanced stages, emphasizing the importance of understanding the resistance mechanisms to develop more effective treatment strategies (1).

The human choriocarcinoma cell line (JAR) exhibits a sphere-like structure and is frequently used to understand the biology of choriocarcinoma in studies of cellular proliferation, differentiation, and trophoblastic malignancy. Despite several studies investigating the role of JAR cells in various biological processes, there is limited information on the distribution and functional roles of stem cell and cancer stem cell markers (2).

Analysis of stem cell markers plays an important role in cancer biology. OCT3/4, CD133, C-KIT (CD117), SALL4 and CD90 (THY1) are important markers associated with pluripotency, cancer stem cells and metastasis (3-7). OCT3/4 is a crucial transcription factor in maintaining pluripotency in embryonic stem cells and is involved in stem cell self-renewal and differentiation. It has also been shown to contribute to maintaining of cancer stem cell properties in various cancers. CD133 is a well-known marker for cancer stem cells in many tumor types, including brain and colon cancers. C-KIT, a proto-oncogene, is involved in tumor progression and especially metastasis of breast and prostate cancers, where they are mutated and associated with aggressive disease (8-9). SALL4, a transcriptional factor, is recognized for its role in promoting metastasis and enhancing the invasive ability of cancer cells through pathways such as TGF-β/SMAD (4, 10, 11). Furthermore, SALL4 expression is also linked to negative prognosis in various cancer contexts, including gastric and colorectal cancers associated with markers of epithelial-mesenchymal transition (EMT) (12-13). CD90 expression is a marker for the establishment of cancer stem cells and

promotes tumor initiation, progression and metastases by modulating cell adhesion and signalling pathways (14-16). Taken together, these markers provide strong evidence for the interdependent nature of tumor self-renewal, promoting cancer progression and malignancy. In light of this information, the expression and distribution of markers in JAR cells are critical to gainining important insights into trophoblastic malignancy and stem cell biology.

However, there is a lack of information in the literature regarding the expression of these markers in the JAR cell line. This is important for studies on choriocarcinoma biology and resistance mechanisms (17). This research aimed to analyze the distribution of key stem cell and cancer stem cell markers, including OCT3/4, CD133, C-KIT, SALL4, and CD90, in the JAR cell line. Investigating the expression of these markers in the JAR cell line is aimed at providing new information about trophoblastic malignancy and its potential to carry stem cell properties (18).

MATERIAL AND METHODS

Cell Culture

JAR (HTB-144) and endometrial epithelial cell line RL95-2 (CRL-1671) were obtained commercially from the American Type Culture Collection (ATCC). JAR cells were cultured in RPMI 1640 (11875093, Gibco) and RL95-2 cells were cultured in DMEM:F12 (Biosera, LM_D12221500) culture medium with 10% fetal bovine serum (FBS) (26140079, Gibco), 1% penicillin-streptomycin (15140148, Gibco) and 1% L-glutamine (25030081, Gibco). After the cells reached 80% confluence, the culture medium was removed and Trypsin-EDTA (T4049, Sigma-Aldrich) was added. After incubation at 37□C for 10 minutes, the cells were collected in 5 ml of culture medium and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed, and the cells were suspended in new culture medium and seeded in a 24-well plate with a round coverslip for immunocytochemistry study.

Colony Forming Assay

A colony formation assay was performed to evaluate the clonogenic capacity of JAR cells. Cells were seeded in a 6-well plate at a density of 500 cells per well and cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin and 1% L-glutamine. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 10 days (ESCO, CCL-170B-8).

Colonies were observed directly using a phase-contrast microscope (Olympus, IX71) to avoid any interference from fixation or staining. Images were taken at regular intervals, and colonies were defined as clusters containing >50 cells. Quantification was performed by manual counting of colonies in phase-contrast images, providing an accurate assessment of colony-forming potential (19- 20).

Immunocytochemistry

JAR and RL95-2 cells were fixed in 4% paraformaldehyde (158127, Sigma-Aldrich) prepared with phosphate buffered solution (PBS, P4417, SentezLab) for 30 min at 4 °C. Cells were treated with 0.01% Tween 20 (P1379, Sigma Aldrich) for 15 min for permeabilization. After washing with PBS, endogenous peroxidase activity was inhibited by 3% hydrogen peroxide (H2O2, 7722-84-1, Santa Cruz) for 5 min at room temperature. Cells were then washed with PBS and incubated with anti-OCT3/4 (A1759, R&D), anti-CD133 (MA5-44377, Thermo Fisher), anti-C-KIT (ab283653, Abcam), anti-SALL4 (#720030, Thermo Fisher), and anti-CD90 (ab307736, Abcam) at 4°C overnight at a dilution ratio of 1:250 for all primary antibodies.

Biotinylated secondary antibody in the Histostain-Plus IHC Kit (HRP, 859043, ThermoFisher) was dropped onto the slides and incubated for 10 minutes. Diaminobenzidine (DAB, 8059, Cell Signalling) was used as chromogen to show immunoreactivity and incubated for 5 minutes.

The samples were washed once with PBS and 3 times with distilled water and counterstained with Mayer haematoxylin (109249, Merck Millipore) for 1 minute. After washing 3 times with distilled water, the slides were covered with closing solution (109016, Merck Millipore). The slides were then examined under a light microscope (Olympus BX40, Tokyo, Japan).

Immunocytochemistry staining intensity for CD133, C-KIT, CD90, OCT3/4, and SALL4 markers was measured using the H-SCORE formula: H-SCORE = Σл(i + 1). Where i represents the staining intensity (1 = weak, 2 = moderate, 3 = strong) and л represents the percentage of cells stained at each intensity level (from 0 to 100%) (21). Intensity levels were categorised as follows:

Weak (1): Light brown staining visible under a microscope.

Moderate (2): Brown staining of medium intensity, clearly distinguishable from the background.

Strong (3): Dark brown staining indicating high marker expression.

H-SCORE provides a semi-quantitative assessment of markers by combining intensity and distribution.

Statistical Analysis

Normality tests of the experimental data were performed using the Shapiro-Wilk test. Parametric data were analysed using a one-way ANOVA test and then compared between the selected groups. Statistical comparisons were analysed using Sidak post hoc test. Non-parametric data were analysed using Kruskal-Wallis test followed by a post hoc Dunn's test. Data are presented as mean ± SEM. GraphPad Prism software version 9 was used for all statistical analyses, and statistical significance was set at p-value < 0.05.

RESULTS

JAR choriocarcinoma cells were epithelioid and demonstrated a colony-forming growth pattern in 2D culture medium. However, the endometrial epithelial cell line RL95-2 cells showed fibroblastic growth without colony formation (Figure. 1).

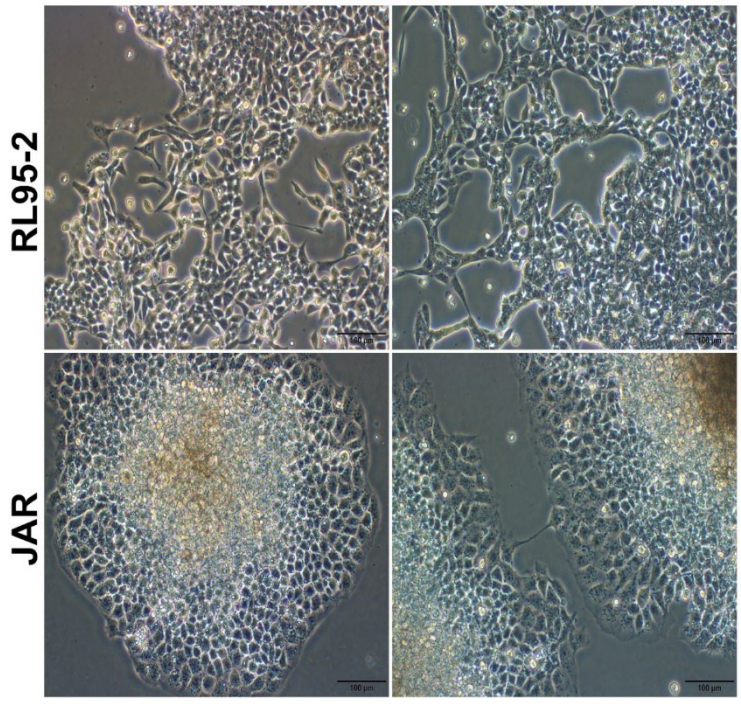

Figure 1. Cell culture photographs of RL95-2 and JAR cell lines. Scale bars: 100 µm.

The colonies formed in JAR cells were visualised by phase-contrast microscopy on days 1, 7 and 10 of culture and analysed by colony formation assay (Figure. 2).

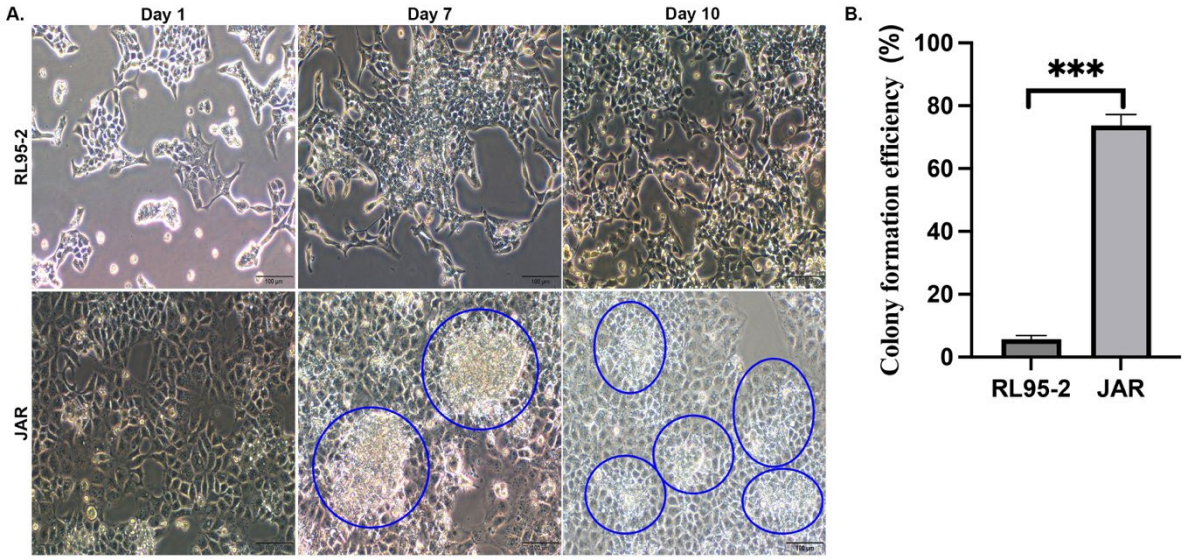

Figure 2. Phase-contrast image of colony formation on days 1, 7, and 10 of culture in RL95 and JAR cells. Scale bars: 100 µm (A). Statistical analysis of colony formation efficiency on day 10 of culture in RL95-2 and JAR cells (B).

The first colony formation in JAR cells was observed on the 7th day of culture, and the colony formation increased on the 10th day of culture (Figure 2A). However, no colony formation was observed in RL95-2 cells during the culture period (Figure 2A).

Colony formation efficiency was statistically higher in JAR cells compared to RL95-2 cells (Figure 2B).

Immunohistochemical analyses revealed that CD133, C-KIT, CD90, OCT3/4, and SALL4 immunoreactivities were negative in RL95-2 cells (Figure 3, Table 1).

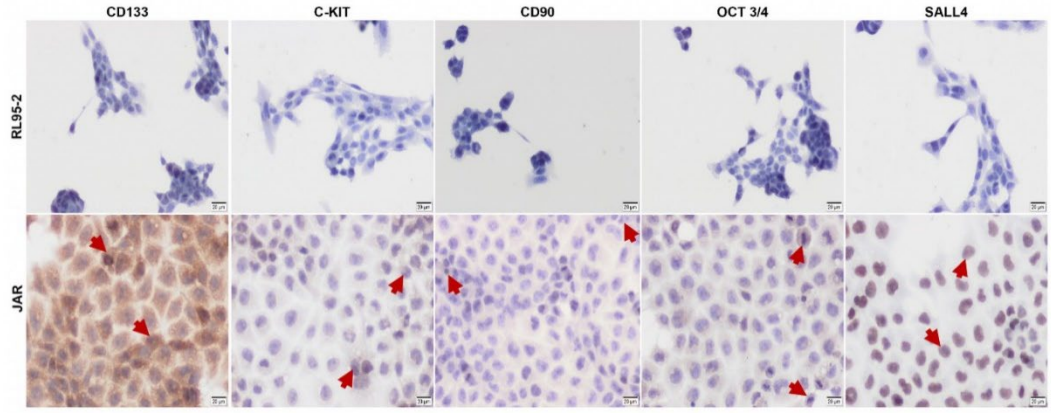

Figure 3. Immunohistochemical staining of CD133, C-KIT, CD90, OCT3/4 and SALL4 in RL95-2 and JAR cells. Red arrows indicate the staining areas of each antibody. Scale bars: 20 µm.

Table 1. H-SCORE results of CD133, C-KIT, CD90, OCT3/4 and SALL4 proteins in RL95-2 and JAR cells.

	CD133	**C-KIT**	**CD90**	**OCT3/4**	**SALL4**
RL95-2 cells	0	0	0	0	0
JAR cells	396.7 ± 2.887	230 ± 5.0	125 ± 8.05	275 ± 5.0	316.7 ± 5.774

In JAR cells, CD133 immunoreactivity was found to be strongly positive. Moreover, CD133 staining pattern was determined to be cytoplasmic (Figure 3, Table 1). In JAR cells, C-KIT immunoreactivity was weakly positive, and the staining pattern was cytoplasmic (Figure 3, Table 1). In JAR cells, CD90 immunoreactivity was weak, and the staining pattern was cytoplasmic and membranous (Figure 3, Table 1). OCT3/4 immunoreactivity in JAR cells was observed to be weakly intense, and the staining pattern was determined to be nuclear (Figure 3, Table 1). SALL4 immunoreactivity suggesting that it plays a role in the regulation of embryonic stem cells was found to be of moderate intensity in JAR cells, and the staining pattern was nuclear (Figure 3, Table 1).

DISCUSSION

Our study revealed that the colony-forming capacity of JAR cells, a choriocarcinoma cell line, is a critical indicator of biological properties such as proliferation ability, tumorigenic potential, cancer stem cell properties, and resistance to therapy. JAR cells have been characterized for their ability to form colonies, which is closely related to the properties of cancer stem cells. In particular, the capacity for self-renewal and differentiation is a hallmark of cancer stem cells, and JAR cells have been shown to exhibit these characteristics. These findings are consistent with the literature (22).

The markers OCT3/4, CD133, C-KIT, SALL4 and CD90 have been extensively studied in various cellular contexts, particularly concerning their role in cancer stem cells, tumor progression, and therapeutic resistance. OCT3/4 is a transcription factor critical for maintaining pluripotency in embryonic stem cells. Its expression is associated with stem cell self-renewal and differentiation and has been shown to play a role in several cancers, where it helps maintain cancer stem cell properties. For example, OCT3/4 expression in breast cancer is associated with aggressive tumor behaviour and poor prognosis (22). CD133 is a well-known marker for cancer stem cells in multiple tumor types, including brain and colon cancers. Since CD133+ cells show greater self-renewal capacity and resistance to conventional

therapies, their expression is associated with enhanced tumor formation and metastatic potential (12- 23). C-KIT expression is associated with poor prognosis and resistance to treatment in hematological malignancies, especially in acute myeloid leukemia. Its role in solid tumors such as gastrointestinal stromal tumors (GISTs) further emphasizes its importance in cancer biology (23). SALL4 is a transcription factor that has emerged as a critical player in various cancers, including gastric and ovarian cancers. It is associated with stem cell regulation and epithelial-mesenchymal transition (EMT) and promotes invasion and metastasis (4-12). SALL4 expression is often associated with aggressive tumor characteristics and poor patient outcomes, making it a potential therapeutic target (24-25). CD90 expression is associated with increased tumor aggressiveness in various cancers, including glioblastoma and nasopharyngeal carcinoma. CD90+ cells often exhibit increased migratory and invasive capabilities. This contributes to tumor progression (26).

The present study has revealed that JAR cells express high levels of CD133 and SALL4, which are associated with enhanced invasive behavior and proliferation. These findings are similar to those observed in other malignancies (4-24). OCT3/4 expression in JAR cells suggests a mechanism by which these cells avoid differentiation and retain their malignant phenotype. Furthermore, the presence of C-KIT indicates that there is a way in which JAR cells can support their growth and survival in the tumor microenvironment, which is in agreement with studies linking C-KIT expression to poor prognosis in various cancers (12-23). The role of SALL4 in JAR cells is particularly noteworthy because it has been shown to regulate cell proliferation and invasion, reinforcing its importance as a therapeutic target (24-25). In addition, CD90 expression in JAR cells suggests that these cells may use CD90-mediated pathways to facilitate tumor progression and metastasis. This finding is consistent with the previous study demonstrating that CD90+ cells exhibit increased levels of aggressiveness in a range of cancer types (26).

The expression of these markers in the JAR cell line used to study choriocarcinoma suggests their potential importance in understanding the biology of this malignancy. The presence of OCT3/4, CD133, C-KIT, SALL4, and CD90 in JAR cells suggests that these cells may exhibit characteristics similar to cancer stem cells and may contribute to their invasive ability and therapeutic resistance.

In summary, the expression of OCT3/4, CD133, C-KIT, SALL4 and CD90 in the JAR cell line highlights the complexity of choriocarcinoma biology and reveals the potential of these markers as therapeutic targets. The findings from our study not only contribute to the understanding of the molecular mechanisms driving choriocarcinoma but also pave the way for future research aimed at developing targeted therapies that may improve patient outcomes.

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