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Stimulation by Exosomes Under Hypoxia Enhances Tumorogenic Properties of Primary and Metastatic Colon and Breast Cancer Cells

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Abstract

BACKGROUND/AIMS: Exosomes are small vesicles that transport bioactive molecules between cells and play a key role in tumor progression. Hypoxic conditions in tumors promote processes such as angiogenesis, metastasis, and drug resistance, primarily through hypoxia-inducible factors (HIF)-1α and HIF-2α. This study investigates exosome production and stemness properties of cancer cells under hypoxic conditions, in primary and metastatic breast and colon carcinoma cell lines.

MATERIALS AND METHODS: Primary breast (MCF7) and colon (Colo320), as well as metastatic breast (M4A4) and colon (Colo741) cancer cell lines, were cultured under hypoxic (3% O_2) and normoxic conditions. Exosomes were isolated using the miRCURY™ Exosome Isolation Kit. The distribution of CD9, CD63, CD133, and HIF-1α was analyzed by immunohistochemical staining, and total miRNA was quantified by NanoDrop.

RESULTS: Under hypoxia, CD63 expression increased in primary cell lines (MCF7 and Colo320) compared to metastatic lines (M4A4 and Colo741). CD133 immunoreactivity was strong in all cells under hypoxia, while HIF-1α levels remained similar across all conditions. Total miRNA levels in Colo320 and Colo741 were unchanged under hypoxia but increased in MCF7 and decreased in M4A4 under hypoxia.

CONCLUSION: Hypoxia significantly enhances exosome secretion and may increase the stemness properties of primary breast and colon cancer cells. Exosomes in the tumor microenvironment could contribute to the malignancy and metastatic potential of aggressive tumors.

Keywords: Exosome, hypoxia, cancer cells, cell differentiation

INTRODUCTION

During tumor development and progression, stromal and cancer cells often have limited access to nutrients and oxygen, a phenomenon known as tumor hypoxia. Hypoxia is a critical factor in promoting cancer progression by causing resistance to radiotherapy and chemotherapy, as well as enhancing the metastatic potential of cancer cells.[1] Hypoxia drives tumor cells to adapt to low oxygen conditions primarily through the activation of hypoxia-inducible factors (HIFs), particularly HIF-1α and HIF-2α, which are responsible for initiating survival pathways under stress.[2,3] These transcription factors not only promote angiogenesis and metabolic reprogramming but also contribute to cancer cell survival in harsh microenvironments.[4] The tumor microenvironment (TME) comprises not only cancer cells but also various stromal components such as fibroblasts, immune cells, extracellular matrix proteins, and secreted factors.[5] This dynamic

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environment plays a crucial role in tumor progression, invasion, and immune evasion.[6] Cancer stem cells (CSCs), a subpopulation of cancer cells within the TME, have been shown to contribute significantly to tumor initiation, metastasis, and resistance to therapies. CSCs maintain their stemness properties within the TME due to the supportive interactions with both cellular and physical factors.[7] Colorectal cancer, the second leading cause of cancer-related deaths in Western countries, represents a prime example of the complex interactions between tumor cells and their microenvironment. These interactions stimulate tumor growth, progression, and metastasis, as well as the development of treatment resistance.[8] Breast cancer, the most commonly diagnosed cancer among women, is influenced by a variety of risk factors, including genetic mutations, reproductive history, and lifestyle factors.[9,10] Early detection and personalized treatment strategies are critical for both colon and breast cancer. Exosomes, small extracellular vesicles (30-100 nm), have garnered attention as potential biomarkers due to their unique molecular cargo, which includes proteins, miRNAs, and lncRNAs.[11] Exosomes differ from other extracellular vesicles in their biogenesis and lipid composition, and they are secreted by various cell types into bodily fluids like blood, saliva, and lymph.[12] The analysis of exosome content from cancer cells provides insights into tumor biology, enabling the identification of novel diagnostic and therapeutic targets.[13]

In cancer, exosome-mediated signaling contributes to tumor progression by facilitating communication between tumor cells and the surrounding stroma. This promotes processes such as angiogenesis, invasion, metastasis, and immune evasion.[14,15] Hypoxia has been shown to increase exosome secretion, and the content of these exosomes often reflects the hypoxic state of the tumor, further influencing tumor behavior.[16] In this study, we aim to analyze the presence and content of exosomes in cell culture media under hypoxic and normoxic conditions in primary and metastatic breast and colon carcinoma cell lines, and to evaluate their potential role in promoting stemness properties in cancer cells.

MATERIALS AND METHODS

Cell culture: Primary colon adenocarcinoma (Colo320, HTL95027, Interlab Cell Line Collection, Genova, Italy) and metastatic colon adenocarcinoma (Colo741, HTL95008, Interlab Cell Line Collection, Genova, Italy) cell lines were cultured in RPMI-1640 (F-1213, Biochrom, Berlin, Germany) supplemented with 10% fetal bovine serum [(FBS), S 0113, Biochrom, Berlin, Germany], 2 mM L-glutamine (K 0283, Biochrom, Berlin, Germany), and 1% penicillin-streptomycin. Similarly, breast cancer cell lines, primary (MCF7, HTB-22, ATCC) and metastatic (M4A4, CRL-2914, ATCC), were cultured in Dulbecco's Modified Eagle Medium containing 10% FBS, 1% penicillin-streptomycin, and 2 mM L-glutamine. All cell lines were incubated at 37 °C with 5% CO_2 in both normoxic and hypoxic (3% O_2) conditions. Each experimental condition was performed in triplicate to ensure reproducibility and reliability of the results.

Statistical Analysis

Immunocytochemical analysis: Cells in all groups were fixed with 4% paraformaldehyde and then incubated with 3% H_2O_2 (Merck, K31355100 303) for 15 min. After washing, permeabilization was performed with 0.1% Triton-X100 (Applichem, Mt 646). The blocking solution (Invitrogen, 859043) was applied, followed by the addition of primary antibodies against CD63 (Santa Cruz Biotechnology, sc-5275), CD9 (Santa Cruz Biotechnology, sc-13118), HIF1α (GeneTex, GTX127309), and CD133 (BiorBy, orb99113) and were incubated overnight at 4 °C. After washing, secondary antibody (Invitrogen, 859043) and diaminobenzidine chromogen (ScyTek, ACC125) were applied. They were stained for 3 minutes with Mayer's hematoxylin (05-06002/L) and then examined under a light microscope (Olympus BX31).

Exosome derivation: *After collecting culture medium from all groups, the miRCURY™ Exosome Isolation Kit (Exiqon 300102) was used for the exosome derivation according to the manufacturer's protocol.*

RNA derivation: Lysis buffer (600 µL) was added to the cell pellets and incubated at room temperature for 5 min. Then 70% ethanol (1:1 ratio with lysis buffer) was added, the mixture was vortexed, and transferred to the column. After centrifugation, 50 µL of elution buffer was added. At the end of the other centrifugation, the amount of RNA was measured with a NanoDrop spectrophotometer.

RESULTS

Semi-adherent adhesion feature of Colo320 (Figure 1A, B), epithelial-shaped feature of Colo741 (Figure 1C, D), fusiform structure of M4A4 (Figure 1E, F) and adherent epithelial feature of MCF-7 (Figure 1G, H) were the same after culturing under both hypoxic (Figure 1A, C, E, G) and normoxic (Figure 1B, D, F, H) conditions. HIF1α immunoreactivity was detected as very weak in both hypoxic and normoxic conditions in all types of cells (Figures 2, 3). Very weak CD9 immunoreactivity was observed in Colo320, Colo741, and M4A4, and weak CD9 immunoreactivity was observed in MCF-7, in both hypoxic (Figure 2) and normoxic (Figure 3) conditions. CD63 immunoreactivity was positive in the Colo320, MCF7, and M4A4 cell lines, while it appeared negative in Colo741 cells under both hypoxic (Figure 2) and normoxic (Figure 3) conditions. The immunoreactivity of CD133 was similar for the Colo741 cell line in both normoxic and hypoxic conditions; however, in hypoxic conditions, increased immunoreactivity was detected in the other cell types (Figures 2, 3). After exosome analysis, the total miRNA in hypoxic, and normoxic conditions was similar in the Colo320 and Colo741 cells. The total amount of miRNA in hypoxic conditions in MCF7 cells was found to be higher than that in the normoxic conditions. In M4A4 cells, the amount of total miRNA in hypoxic conditions was less than in normoxic conditions.

DISCUSSION

In this study, exosome production was investigated in both primary and metastatic breast and colon cancer cells under hypoxic and normoxic conditions. Our findings showed a significant increase in exosome production in breast cancer cells under hypoxia, suggesting that hypoxia enhances exosome-mediated communication in the TME, potentially promoting tumor progression. The upregulation of CD63 expression in primary cells further supports the idea that exosome production is heightened in response to hypoxic stress. In contrast, hypoxia had a less pronounced effect on exosome production in colon cancer cells, indicating that these cells may utilize alternative adaptive mechanisms or rely less on exosome-mediated signaling. The differential responses between breast and colon cancer cells to hypoxia highlight the complexity of tumor biology and the distinct ways various cancer types interact with their microenvironment.

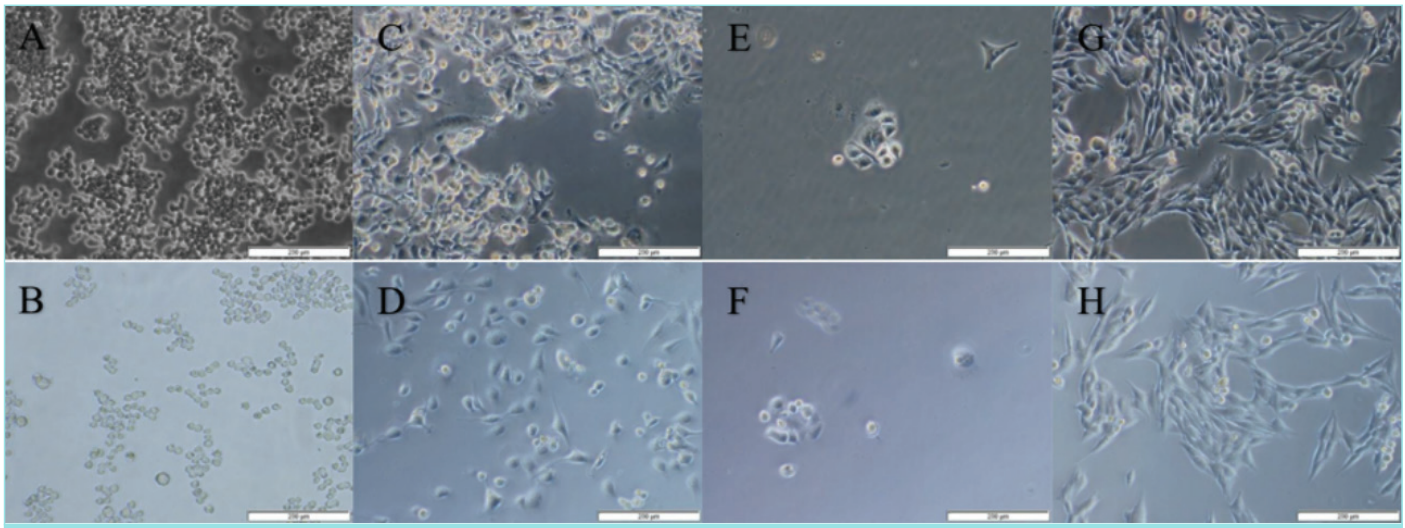


Figure 1. Colo320 (A, B), Colo741 (C, D), MCF7 (E, F) and M4A4 (G, H) cells were cultured in hypoxic (A, C, E, G) and normoxic (B, D, F, H) conditions. Scale bars: 200 µm.

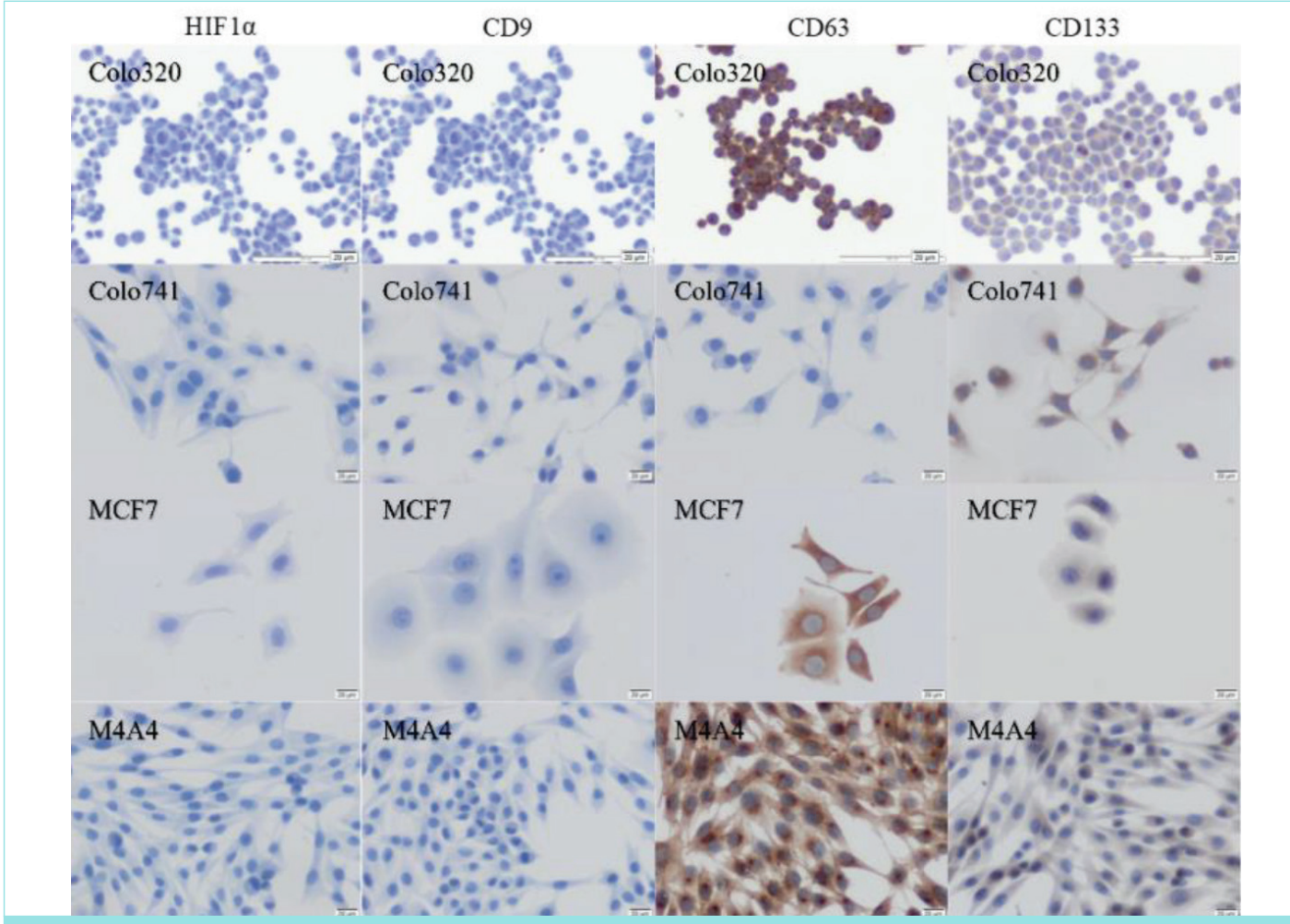


Figure 2. HIF1α, CD9, CD63 and CD133 immunoreactivities of Colo320, Colo741, MCF7 and M4A4 cell lines in hypoxic condition. Scale bars: 20 µm.

HIF: Hypoxia-inducible factors.

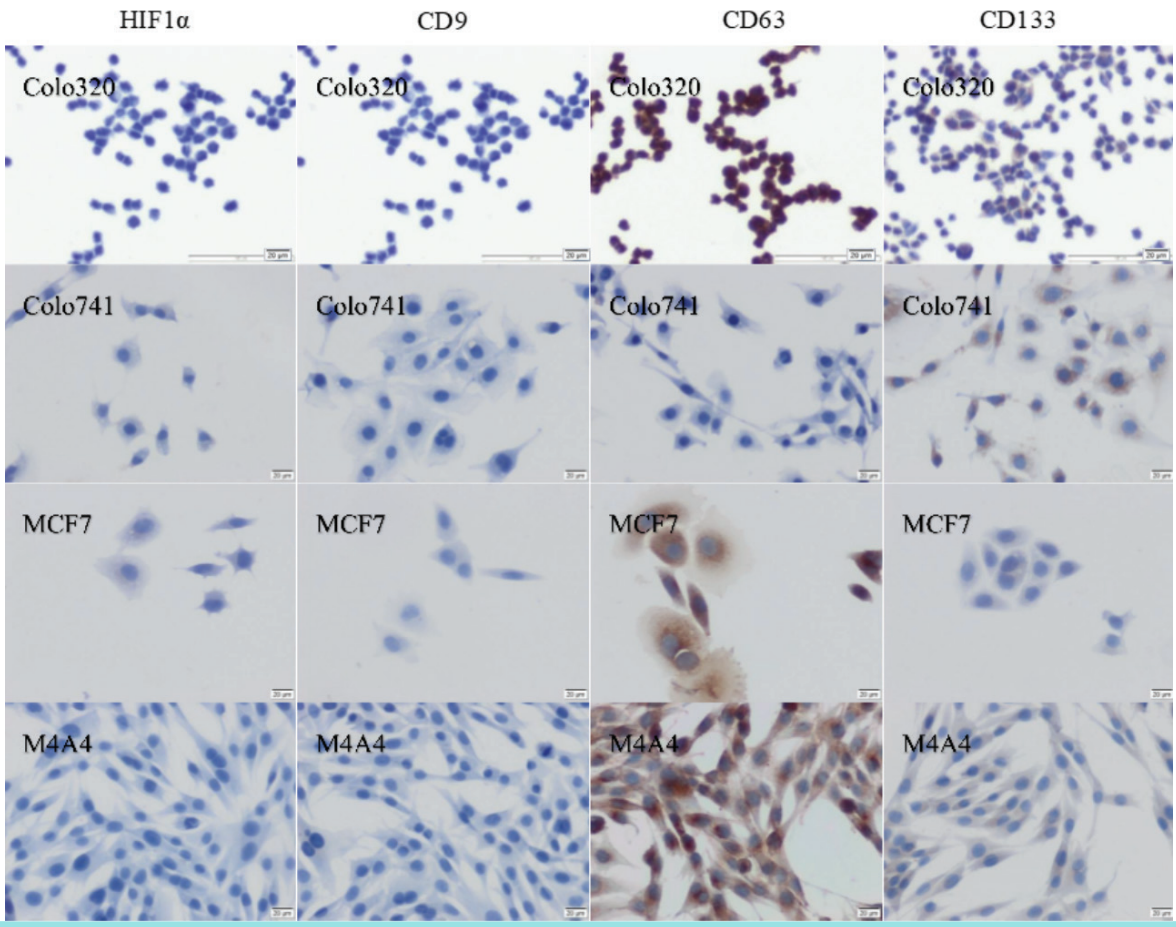


Figure 3. HIF1α, CD9, CD63 and CD133 immunoreactivities of Colo320, Colo741, MCF7 and M4A4 cell lines in normoxic condition. Scale bars: 20 µm.

HIF: Hypoxia-inducible factors.

Moreover, the increase in CD133 immunoreactivity under hypoxic conditions suggests that hypoxia may support CSC properties, which are crucial for tumor initiation and progression.[17] Changes in miRNA profiles in response to hypoxia also imply that exosomes play a role in modulating tumor growth and metastasis through altered miRNA signaling.

CONCLUSION

Overall, this study demonstrates that hypoxia-induced exosome production may influence cancer stemness and tumor cell interactions with the microenvironment. The observed differences in exosome production across cancer types offer valuable insights into cancer biology and may guide the development of personalized therapeutic strategies in the future.

MAIN POINTS

- Hypoxia significantly enhances exosome secretion and may increase the stemness properties of primary breast and colon cancer cells.
- Exosomes from hypoxic conditions show distinct miRNA profiles compared to normoxic conditions, which could impact tumor progression.
- CD63 expression was notably increased in primary cancer cells under hypoxia, while metastatic lines showed less response.
- Exosome-mediated signaling in the tumor microenvironment contributes to malignancy and metastasis, particularly in aggressive tumors.

ETHICS

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: İ.H.G., H.K.E., H.S.V., Design: İ.H.G., H.K.E., H.S.V., Data Collection and/or Processing: İ.H.G., H.K.E., R.K.K., H.S.V., Analysis and/or Interpretation: İ.H.G., H.K.E., R.K.K., H.S.V., Literature Search: İ.H.G., H.K.E., R.K.K., H.S.V., Writing: İ.H.G., H.K.E., H.S.V.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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