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Disseminated Tumor Cells in Bone Marrow in Gastric Cancer Patients with Obesity

Bubnovskaya L[1]*, Ganusevich I[1], Merentsev S[2] and Osinsky D[2]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Ukraine
[2]City Clinical Oncological Center, Ukraine

Corresponding Author: Larissa Bubnovskaya, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Vasilkovskaya Str. 45, Kiev-03022, Ukraine; **E-mail:** osinskysp12@ukr.net

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Background

Obesity is a risk factor for cancer development and is associated with poor prognosis in multiple tumor types. There is emerging evidence of a strong association between obesity and gastrointestinal cancer. The molecular mechanism underlying gastric cancer invasion and metastasis is still poorly understood. Problem of disseminated tumor cells (DTCs) in gastric cancer remains to be relevant for clinics and less is known concerning this problem for patients with obesity.

Aim

This study was aimed to evaluate how incidence of DTCs in bone marrow is conditioned by excess of adipocites in tumor microenvironment of patients with gastric cancer and obesity.

Results

There was not found the associations between availability of DTCs in BM as well CXCR4-positive cells in tumor and body mass index (BMI) but incidence of DTC in BM was associated with high density of cancer-assocsated adipocytes (CAAs) as well with high number of CXCR4-positive cells in tumor of patients with BMI<25 and BMI>25<30 but it was not true for patients with BMI>30 where frequency of DTCs finding in BM was significantly decreased and that was statistically significant.

Conclusion

In patients with BMI>30 high density of CAAs and high number of CXCR4-positive cells in tumor may create specific tumor microenvironment that prevent tumor cells to leave primary lesion.

Obesity is associated with poor prognosis in multiple tumor types [1]. There is emerging evidence of a strong association between obesity and gastrointestinal cancer [2]. In contrast to the convincing evidence that obesity (measured by body mass index, BMI) increases the risk of many different types of cancer, there is an ambiguity in the role of obesity in survival among cancer patients [3, 4]. Some studies suggested that higher BMI decreased mortality risk in cancer patients, a phenomenon called the obesity paradox [1]. Changes that occur in the obese state and the biologic mechanisms underlying the connections of these changes to increased cancer risk are poorly understood [5–6]. Many types of solid tumors grow in proximate or direct contact with adipocytes and adipose-associated stromal and vascular components. During interaction with cancer cells adipocytes dedifferentiate into pre-adipocytes or are reprogrammed into cancer-associated adipocytes (CAAs) that modulate the tumor microenvironment by promoting angiogenesis, affecting immune cells and altering metabolism to support growth and survival of metastatic cancer cells [7]. Quail D et al. indicate that special consideration of the obese patient population is critical for effective management of cancer progression [8].

During tumor progression, cells can acquire the capability for invasion and metastasis to escape the primary tumor, first of all, from breast, lung, colorectal and prostate, and colonize new organs [9, 10]. Tumor cells leaving primary site can settle mainly in bone marrow (BM) as a common homing-organ for disseminated tumor cells (DTCs) with potency to form the metastases [11–13]. The most important factors controlling cellular migration are chemokines and their receptors. Stem cell receptor CXCR4 as a transmembrane chemokine receptor and its specific ligand CXCL12 (stromal cell-derived factor 1, SDF-1α) play a vital role in dissemination of tumor cells from primary sites, transendothelial migration as well as homing of cancer stem cells. In the tumor microenvironment under hypoxic condition cells of a growing tumor are reprogrammed to express the CXCR4 receptor thereby enhancing the metastatic potential of the tumor cells. [14–17]. The molecular mechanism underlying gastric cancer invasion and metastasis is still poorly understood. Problem of DTCs in gastric cancer remains to be relevant for clinics [18] and less is known concerning this problem for patients with overweight and obesity [19]. Therefore our study was aimed to evaluate how of CAA density, CXCR4 expression in primary tumor affect presence of DTCs in BM of patients with gastric cancer according to the body mass index (BMI).

Patients and Methods

Patients

A total of 94 patients (60 men and 34 women) with primary gastric cancer were diagnosed and treated at the City Clinical Oncological Center (Kiev). No patient received any pre-operative anti-cancer therapy. Tumors were classified and staged according to the 2002 version of the UICC staging system [20]. Histological types of tumor were evaluated by WHO histological classification (2000) [21]. Tissue samples were taken immediately after tumor excision. Preoperatively, 2.0–3.0 ml of BM aspirates from the sternum with conventional cautions to avoid the hit of skin epithelial cells into the sample were obtained. All patients were thoroughly informed about the study that was approved by the local ethics committee.

Immunocytochemical Examination of Bone Marrow

Detection of tumor cells (cytokeratin-positive cells, CK-positive cells) in BM cytospin preparations fixed in acetone was provided by APAAP method (alkaline phosphataseantialkaline phosphatase) and visualization system EnVision G/2 System/AP Rabbit/Mouse (Permanent Red) (Dako Cytomaiton, Denmark). Monoclonal mouse antibodies against panCK (clone AE1/AE3, Dako Cytomation, Denmark) were used as primary antibodies. Each assay was controlled negatively by staining of one cytospin preparation with nonspecific IgG1 (MOPC21, Sigma). Number of tumor cells (CK-positive cells) was expressed on 10^6 BM mononuclear cells. BM samples were scored "positive" if the presence of two or more CK-positive cells per 10^6 mononuclear cells were detected (from 6 to 12 slides per patient were screened).

Immunohistochemical Examination of Tumor Tissue

Expression Perilipin ($Plin5^+$) as a marker for viable adipocytes as well expression of CXCR4 were provided on deparaffinized slides using specific polyclonal rabid antibodies (Perilipin-5/OXPAT Antibody, Termoscientific, USA) dilution 1:200 and specific monoclonal mouse antibodies: clone AB2074 (Abcam, UK), respectively. Slides for evaluation of $Plin5^+$ were covered with 1% of bovine serum albumin (BCA) and incubated with polyclonal antibodies during for 1hour and then washed in phosphate-buffered saline (PBS). Immunoreactions were detected and visualized with the polymer-peroxidase method (EnVision+/HRP and 3, 3-diaminobenzidine; DakoCytomation, Denmark) followed by counterstaining with Mayer hematoxylin. Negative control was employed in which the primary antibody was replaced by phosphate-buffered solution (PBS). Immunopositive cells were counted per 1000 cells in each slide and the number of positive cells was reported as percent. When the tumor consisted of more than 10% of CXCR4-positive cells, the case was scored as positive.

Body mass index (BMI, kgm^{-2})

Patients were classified according to BMI, following the WHO definitions, as underweight, normal (18.5–<25.0 kg/m^2), overweight (25.0–<30.0 kg/m^2) or grade 1 obesity (30.0–<35.0 kg/m^2).

Statistical Analysis

All statistical analyses were conducted using the NCSS 2000/PASS 2000 and Prism, version 4.03 software packages. Prognostic values of relevant variables were analyzed by means of the Cox proportional hazards model using Odds ratio and χ^2 test. Two-tailed P values <0.05 were considered statistically significant.

Results

CAAs in Tumors of Patients According to BMI

Individual patient data from a total 94 histological confirmed gastric cancer patients were included in this study. Median number of CAAs in tumors was 26.5%. We defined this number as the cut-off value and classified all cases into high- or low-density groups. Overall, 48.4% of tumors were characterized by a low density of CAAs and 51.6% by high CAAs during follow-up. 39.5%, 46.4%, 89.5% of patients with BMI<25, BMI>25<30, BMI>30, respectively, had high CAAs in tumors. The probability of availability of high density of CAAs in tumor of patients with BMI>30 is increased by a factor of almost 9 (OR 8.84, χ^2 = 13.47, 95%CI 16.777–4.665, P<0.01) as compared with BMI<30. Data obtained demonstrate that adipocytes are as major component of the microenvironment of gastric cancer, especially under obesity.

CK-positive Cells in Bone Marrow

Overall, 88.3% of patients have been with M_0 category. It was determined that CK-positive cells were detected in BM of 50.1% gastric cancer patients among all investigated. There was no association between DTCs in BM and clinicopathological characteristics. It makes no difference between of groups of patients according to BMI concerning the availability of DTCs in BM: 47.6%, 56.2% and 41.2% of patients with BMI<25, BMI>25<30, BMI>30 had DTCs in BM, respectively. Meanwhile, it was found the association between the presence of DTCs in BM and density of CAAs in tumors: DTCs in BM were detected in 41.3% and in 58.7% of patients when tumors characterized by low and high density of CAAs, respectively. When tumors characterized by high density of CAAs appearance of tumor cells in BM has been found in 35.7% of patients with BMI>30 as compare with 70.6% and 62.5% of patients with BMI<25 and BMI>25<30. In patients with obesity frequency of DTCs finding in BM was significantly decreased and it was statistically significant (OR 4.33, χ^2 = 3.82, 95%CI 9.341–2.007, P<0.05) as compare with patients with BMI<25. It may be suggested that adipocites, namely CAAs, playing an essential role in the regulation of metabolic functions in the variety of processes involved in metastatic spread of tumor cells.

CXCR4-positive Cells in Tumor Tissue

Overall, 83.1% of patients had tumors with CXCR4-positive cells. Statistically significant correlation between CXCR4-positivity of tumors and clinical characteristics was not found. The median number of $CXCR4^+$ cells was 24.2% (range of 13.4–81.0%). It makes no difference between of groups of patients according to BMI concerning the CXCR4-positive cells: 66.7%, 65% and 60% of patients with

BMI<25, BMI>25<30, BMI>30 had high number of CXCR4-positive cells in tumor, respectively. Meanwhile, it was found the association between high number of CXCR4-positive cells and density of CAAs in tumors. High number of CXCR4-positive cells were detected in 35.3% and in 72.7% of patients when tumors characterized by low and high density of CAAs, respectively (OR 7.3, χ^2 = 12.45, 95%CI 13.654–4.208, P<0.01).

The mean number of CXCR4-cells in tumors with high density of CAAs was 47.4±1.9%, 37.8±4.1% and 48.5±2.9% in patients with BMI<25, BMI>25<30, BMI>30, respectively.When tumors characterized by high density of CAAs presence of high number of CXCR4-positive cells have been found in 77.8%, 62.5% and 71.4% of patients with BMI<25 and BMI>25<30 and. BMI>30, respectively, and presence of DTCs in BM in these groups of patients was the following: 88.9% of patients with BMI<25, in 58.3% of patients with BMI>25<30 and in 18.2% of patients with BMI>30.It is notably important to note that in patients with BMI>30 having high density of CAAs and high number of CXCR4-positive cells in primary tumor the incidence of DTCs in BM was rather low. It may be supposed that under obesity additional mechanisms may be switched off in tumor microenvironment modulated by excess of adipocites to prevent cells escaping.When tumors characterized by low density of CAAs low number of CXCR4-positive cells was detected in 33.3%, 51% and 44.1% of patients with BMI<25, BMI>25<30 and BMI>30, respectively. Presence of DTC in BM in these groups of patients was the following: in 31.5% of patients with BMI<25, in 44.6% of patients with BMI>25<30 and in 41.1% of patients with BMI>30.

Conclusion

There was not found the associations between availability of DTCs in BM as well CXCR4-positive cells in tumor and BMI but incidence of DTC in BM and high number of CXCR4-positive cells in tumor associated with high density of CAAs of patients with BMI<25 and BMI>25<30 but it is not true for patients with BMI>30 where frequency of DTCs finding in BM was significantly decreased and that is statistically significant.In patients with BMI>30 high density of CAAs and high number of CXCR4-positive cells in tumor may create specific tumor microenvironment that prevent tumor cells to leave primary lesion. Understanding the metabolic changes that occur in obese individuals may also help to elucidate more effective treatment options for these patients when they develop cancer**.**

References

1. Wang J, Yang DL, Chen ZZ, Gou BF (2016) Associations of body mass index with cancer incidence among populations, genders, and menopausal status: A systematic review and meta-analysis. *Cancer Epidemiol* 42: 1–8.
2. Donohoe CL, Pidgeon GP, Lysaght J, Reynolds JV (2010) Obesity and gastrointestinal cancer. *Br J Surg* 97: 628–642.
3. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008) Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 37: 569–578.
4. Iyengar NM, Gucalp A, Dannenberg AJ, Hudis CA (2016) Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. *J Clin Oncol* 34: 4270–4276.
5. Renehan AG, Zwahlen M, Egger M (2015) Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat Rev Cancer* 15: 484–498.
6. Hopkins BD, Goncalves MD, Cantley LC (2016) Obesity and Cancer Mechanisms: Cancer Metabolism. *J Clin Oncol* 34: 4277–4283.
7. Nieman KM, Romero IL, Van Houten B, Lengyel E (2013) Adipose tissue and adipocytes support tumorigenesis and metastasis. *Biochim Biophys Acta* 10: 1533–1541.
8. Quail D, Olson OC, Bhardwaj P, Walsh LA, Akkari L, et al. (2017) Obesity alters the lung myeloid cell landscape to enhance breast cancer metastasis through IL5 and GM-CSF. *Nat Cell Biol* 19: 974–987.
9. Gupta GP, Massagué J (2006) Cancer metastasis: building a framework. *Cell* 127: 679–695.
10. Valastyan S, Weinberg RA (2011) Tumor metastasis: molecular insights and evolving paradigms. *Cell* 147: 275–292.
11. Pantel K, Alix-Panabières C, Riethdorf S (2009) Cancer micrometastases. *Nat Rev Clin Oncol* 6: 339–351.
12. Pantel K, Hayes D (2018) Disseminated breast tumour cells: biological and clinical meaning. *Nat Rev Clin Oncol* 15: 129–131.
13. Pantel K, Brakenhoff RH, Brandt B (2008) Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer* 8: 329–340.
14. Sun X, Cheng G, Hao M, Zheng J, Zhou X, et al. (2010) CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression. *Cancer and Metastasis Rev* 29: 709–722.
15. Burger J, Kipps T (2006) CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 107: 1761–1767.
16. Zhang Z, Ni C, Chen W, Wu P, Wang Z, et al. (2014) Expression of CXCR4 and breast cancer prognosis: a systematic review and meta-analysis. *BMC Cancer* 14: 49.
17. Xiang Z, Zhou Z-J, Xia G-K, Zhang XH, Wei ZW, et al. (2017) A positive crosstalk between CXCR4 and CXCR2 promotes gastric cancer metastasis. *Oncogene* 36: 5122–5133.
18. O'Sullivan J, Lysaght J, Donohoe CL, Reynolds JV (2018) Obesity and gastrointestinal cancer: the interrelationship of adipose and tumour microenvironments. *Nat Rev Gastroenterol Hepatol* 15: 699–714.
19. International Union Against Cancer (2002) TNM Classification of malignant tumors, edited by L. H. Sobin and C. Wittekind, Wiley-Liss, New York, NY, USA, 6th edition, 2002.
20. C Fenoglio-Preiser, F Carneiro, P Correa, et al. (2000) "Gastric carcinoma,"in World Health Organization Classification of tumors. Tumours of the Stomach. In: S. R. Hamilton and L. A. Aaltonen (eds.). vol. 3, chapter 3, Pg No: 39–52, IARC Press, Lyon, France, 2000.

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