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Original Article

Tumor cell phagocytosis (cannibalism) in lung cancer: possible biomarker for tumor immune escape and prognosis

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Abstract: Background: Tumor cell phagocytosis (cannibalism) is rarely seen in lung carcinomas. Little is known about its underlying cellular pathogenesis and associated significance as tumor immune escape mechanism. Methodology: The cases of lung cancer diagnosed at department of Pathology, VPCI over 13-year period, 2007-2020 (n = 350) were retrospectively reviewed. The cases displaying cannibalism were correlated with their tumor morphology, coexisting inflammation, patient age at presentation, sex, stage/grade, and smoking status. Results: Cannibalism was identified in 10/350 (2.86%) cases of lung cancer. 9/10 (90%) were males and 1/10 (10%) was female. These patients ranged from 48-71 years of age and presented with history of chest pain, anorexia and weight loss. History of smoking was seen in 9/10 (90%) cases while 10% were non-smokers. Mass lesions were seen on CT scan and CT-guided fine needle aspiration cytology (FNAC) was performed. Cytopathology revealed squamous cell carcinoma (5/10, 50%), adenocarcinoma-3/10 (30%), adenosquamous carcinoma (1/10, 10%), and non small cell lung carcinoma (1/10, 10%). No association with small cell carcinoma was seen in our study. Background inflammation and infiltration of acute on chronic inflammatory infiltrate were seen in 6/10 or 60% cases. Conclusion: Lung cancers rarely show cannibalism, a tumor immune escape mechanism, even in advanced stage. This phenomenon correlates with squamous cell and adenocarcinoma morphology, tumor associated inflammatory infiltrate, and smoking status. It may be considered as a possible biomarker for tumor immune escape and poor prognosis.

Keywords: Cannibalism, lung cancer, tumor cell phagocytosis, tumor immune escape

Introduction

The cell-in-cell phenomenon in tumor cells was first observed nearly a decade ago [1]. This phenomenon of one cell completely interiorizing other cells, leading to death of the interiorized cells and/or complete auto destruction of engulfing cell is called “cannibalism” [2]. The presence of internalized cells in tumor cells has been observed to be related to a poor cancer prognosis [3]. This process is commonly associated with phagocytosis by macrophages which results in the resolution of inflammation, antigen presentation, and disposal of apoptotic cells [4]. This phenomenon has been variably referred to as “cellular phagocytosis” [5], “cell in cell appearance” [6], “cell in cell pattern” [7], “one cell delicately wrapped around the next” [8], or “tumor cell embraced by another tumor cell” [9]. In the lung, “cell phagocytosis”and “phagocytosis of tumor cell by tumor cell” have been identified in giant cell carcinomas [10] and small cell carcinoma lung [11].

In cancers, the occurrence of cannibalism, its underlying pathomechanisms and its biological relevance is a matter of recent investigation. (I) Some recent studies have considered tumor cell cannibalism to be “aberrant phagocytosis”, in order to obtain nutrients, similar to professional phagocytes [12]. (II) Others have suggested that tumor cells engulf tumor-infiltrating lymphocytes and erythrocytes, either dead or alive, depending on their microenvironmental

condition (low nutrient supply or low pH). The malignant cells thus directly and efficiently neutralize the anti-tumor immune response. (III) A third theory considers tumor cell cannibalism representative of cellular regression to an ancestral or primeval life style similar to unicellular microorganisms, such as amoebas [13]. The main goal of all the above theories is tumor cell survival and propagation in an overcrowded and very hostile microenvironment. Targeting cannibalism by metastatic cells and its mediators (TM9 protein-TM9SF4) may represent a novel therapeutic target [13]. Whether cell cannibalism is associated with the inflammatory infiltration and immune escape of lung cancer remains to be answered?

Therefore, we assessed the cannibalism associated with lung cancer and correlated with their tumor morphology, immune cell infiltrate and clinical features as a possible biomarker for tumor immune response and prognosis.

Case history

The cases of lung cancer diagnosed at department of Pathology, VPCI over 13-year period, 2007-2020 (n = 350) were retrospectively reviewed. At the time of presentation and initial diagnosis, these cases were correlated with their tumor morphology, coexisting inflammation, patient age at presentation, sex, stage/grade, and smoking status. None of these lung cancer patients had received prior radiotherapy or chemotherapy.

Cannibalism was identified in 10/350 cases on cytology. 9/10 (90%) were males and 1/10 (10%) was female (**Table 1**). These patients ranged from 48-71 years of age and presented with history of chest pain, anorexia and weight loss. History of smoking was seen in 9/10 (90%) cases while 10% were non-smokers. Mass lesions were seen on CT scan and CT-guided FNAC was performed. Cytopathology revealed squamous cell carcinoma (5/10, 50%), adenocarcinoma-3/10 (30%), adenosquamous carcinoma (1/10, 10%), and non-small cell lung carcinoma (1/10, 10%). No association with small cell carcinoma was seen in our study. Tumor cell phagocytosis of neutrophils (**Figure 1**) and other tumor cells (**Figure 2**) with engulfing cell showing apoptosis of internalized cells arranged in horseshoe pattern (**Figure 3**) were seen. Background inflammation and infiltration of acute on chronic inflammatory infiltrate were seen in 60% (6/10) cases (**Figure 4**).

Discussion

Cannibalism or anthropophagy (from the Greek anthropos “man” and phagein “to consume”) was first used to refer to the act of humans eating other humans [13]. Identification of this phenomenon in tumors was considered as a symbol of malignancy [14-16]. Cannibalism has been seen in many malignancies like urinary bladder carcinoma, malignant melanoma, gastric adenocarcinoma, endometroid adenocarcinoma, malignant mesothelioma, small cell carcinoma lung and rarely in adenocarcinoma lung. Previous studies have suggested that cannibalism can be induced by serum factor(s). Also, the degree of “cannibalism” is observed to be partly dependent on cell density [2]. These serum factor(s) have the potential to induce auto destruction of tumor cells and may be of therapeutic value.

Pathomechanisms

Three different mechanisms of cell-to-cell recognition and cell penetration strategies involving tumor cells have been considered [13, 17] (**Figure 1**). These include: (I) Cannibalism, which is the active internalization and destruction of tumor cells (dead or living) and involves Transmembrane 9 Superfamily Member 4 (TM9SF4), Ezrin, and caveolin-1; (II) emperipolesis which is the phagocytosis of intact hematopoietic cells (neutrophils, lymphocytes and plasma cells, mainly), involves the Ezrin, Lymphocyte function-associated antigen 1 (LFA-1), and Intercellular adhesion molecule-1 (ICAM-1); (III) entosis which is a homogenous live-cell invasion mechanism and resembles host-parasite interaction (active invasion of internalized cell) [18] involving E/P-cadherin, Rho-associated protein kinase (ROCK)-actin/myosin pathway and imbalance in actomyosin contraction. This cell-to-cell interaction is associated with strong epithelial-mesenchymal transitional phenotype of the engulfing cancer cells. The epithelial nature of internalized cells is evaluated by their pancytokeratin (strong) and E-cadherin (weak) positivity [19] with ezrin being expressed on phagocytic vacuoles [20].

Table 1. Clinical/radiological/pathological features of lung cancer cases showing cannibalism

Sr No.	Age/ Sex	Smoking status	CT Scan	CT-Guided FNAC pathology	Background inflammation
1	68/M	tobacco smoker	Right lung mass	Well differentiated Squamous cell carcinoma with tumor giant cells showing engulfed neutrophils in cytoplasm	Moderately dense inflammatory infiltrate comprising of neutrophils, lymphocytes, macrophages
2	50/F	non-smoker	Right lung mass	Papillary adenocarcinoma with numerous loosely cohesive papillary clusters of tumor cells and binucleated tumor giant cells with cannibalism	moderately dense inflammatory cells comprising of plasma cells and alveolar macrophages
3	48/M	current smoker (since 30 month)	Left upper lobe mass with supraclavicular lymph-adenopathy	Adenosquamous carcinoma with papillary clusters of large pleomorphic tumor cells admixed with tumor cells having angulated cytoplasmic margins and inky blue cytoplasm	Tumor giant cells with engulfed neutrophils were seen
4	63/M	tobacco smoker	Right upper lobe mass in posterior-segment measuring 6.8 × 6.2 cm extending from mediastinum to chest wall with rib destruction and lytic lesion in thoracic vertebra	Squamous cell carcinoma with tumor cells showing the intra cytoplasmic engulphed nuclear material	highly dense acute on chronic inflammatory infiltrate
5	71/M	tobacco smoker	Right lower lobe cavitatory mass lesion. Patient developed paraplegia and expired within 10 days	Adenocarcinoma with tumor cells showing neutrophilic phagocytosis	Moderately dense acute on chronic inflammatory infiltrate
6	68/M	tobacco smoker (25 pack years)	Left upper and middle zone non-homogenous opacity in XRC	Adenocarcinoma with neutrophilic phagocytosis by tumor cells	None
7	65/M	tobacco smoker (40 pack years)	Right lower lobe-lobulated heterogenous lesion and right pleural effusion. Left upper lobe-focal pulmonary infiltrates in apico-posterior segment	Squamous cell carcinoma with Tumor giant cells and multinucleated giant cells engulfing neutrophils	moderately dense acute on chronic inflammatory infiltrate comprising of neutrophils, lymphocytes and alveolar macrophages
8	68/M	tobacco smoker	Left lung mass	Squamous cell carcinoma with pleomorphic tumor cells and angulated cytoplasm margin and necrosis	None
9	48/M	tobacco smoker	Left lung mass with carinal lymphadenopathy	NSCLC with pleomorphic vesicular nucleus, non keratanised angulated cytoplasm and atypical mitosis	None
10	60/M	tobacco smoker	Right lung mass	Squamous cell carcinoma with atypical keratanised and non-keratanised squamous epithelial cells	None

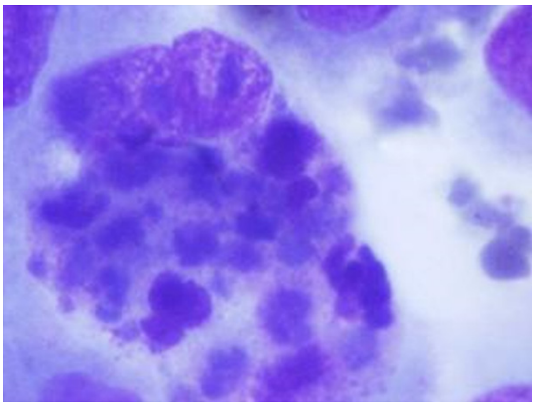

Figure 1. CT guided FNAC smear showing lung cancer with tumor cell phagocytosis of numerous neutrophils. May Grunwald Giemsa stain ×400.

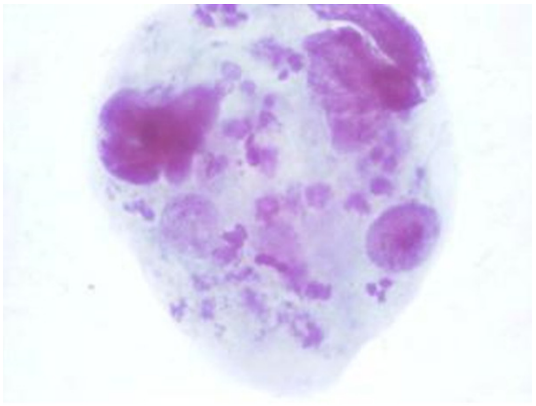

Figure 2. CT guided FNAC smear showing lung cancer with tumor cell phagocytosis of other tumor cells and neutrophils. Papanicoloau stain ×400.

Cell cannibalism: diagnostic marker of malignancy

Tumor cell cannibalism of neutrophils and erythrocytes has been associated with squamous cell carcinoma, small cell carcinoma and melanoma [21]. Cannibalism has been assessed to be a feature of malignancy in effusion and urine cytology [22]. Therefore, cell cannibalism has been considered to be a dependable cytological feature of malignancy in effusion cytology.

Cell cannibalism: prognostic marker of malignancy

The host immune response to tumor cells includes tumor associated macrophage infiltration deep into the tumor, their differential phenotypic activation and recruitment of immune lymphocytes [23]. Tumor cannibalism often involves T lymphocytes and can significantly increase metastatic cancer cell survival [14]. Thus, the tumor cells not only survive in condition of low nutrient supply by eating other live cells, but also eating the live T lymphocytes, which are programmed to kill them and provide them a novel mechanism of tumor immune escape. Thereby indicating that, this cellular phenomenon can be a marker of tumor immune escape, progression and poor prognosis. Thus, we identify cell cannibalism to be associated with the inflammatory infiltration and immune escape of lung cancer.

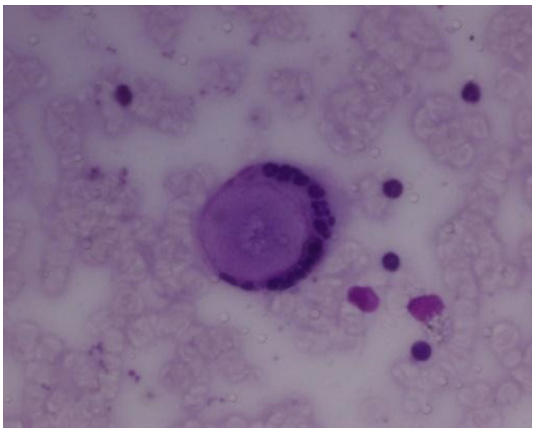

Figure 3. CT guided FNAC smear showing lung cancer with apoptosis of engulfing cell and internalized cells arranged in horse shoe pattern. May Grunwald Giemsa ×400.

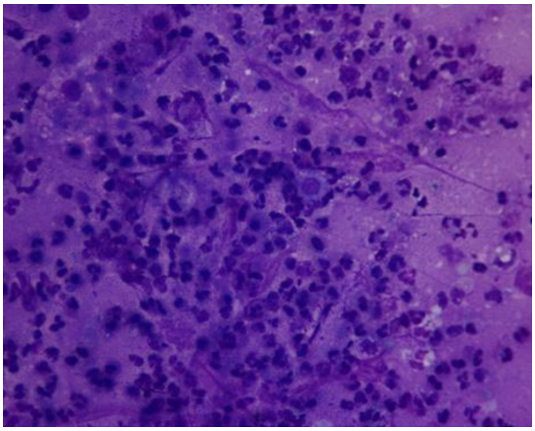

Figure 4. CT guided FNAC smear showing lung cancer with singly arranged tumor cells admixed in dense inflammatory background. May Grunwald Giemsa ×100.

Electron microscopy (EM)

EM studies have identified the sequence of events of cannibalism of live lymphocytes by cancer cells: an early "fusion-like" process, tumor cell invagination and entrapment of live cells. This process significantly differs from phagocytosis, where the extended ruffles and pseudopods are formed and embrace and engulf the external body [24]. On electron microscopy, the engulfed neutrophils present within adenocarcinoma cells show varying morphology. EM changes range from early apoptotic features with perinuclear chromatin aggregation and intact cytoplasm to late apoptotic changes with uniform, collapsed nucleus and tightly packed cytoplasmic granules [3].

Summary and conclusion

Cannibalism can be of clinical significance in lung cancer as diagnostic marker and indicator of tumor progression and poor prognosis. In the present study we demonstrate the rare occurrence of cannibalism in non-small cell lung cancers and its association with smoking, squamous and adenocarcinoma morphology and inflammatory background on histopathology.

Disclosure of conflict of interest

None.

Abbreviations

FNAC, Fine Needle Aspiration Cytology; TM-9SF4, Transmembrane 9 Superfamily Member 4; LFA-1, Lymphocyte Function-Associated Antigen 1; ICAM-1, Intercellular Adhesion Molecule-1; ROCK, Rho-Associated Protein Kinase; EM, Electron Microscopy.

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References

[1] Wang X, Li Y, Li J, Li L, Zhu H, Chen H, Kong R, Wang G, Wang Y, Hu J and Sun B. Cell-in-cell phenomenon and its relationship with tumor microenvironment and tumor progression: a review. Front Cell Dev Biol 2019: 7: 311.

[2] Brouwer M, de Ley L, Feltkamp CA, Elema J and Jongsma AP. Serum-dependent "cannibalism" and autodestruction in cultures of human small cell carcinoma of the lung. Cancer Res 1984; 44: 2947-2951.

[3] Caruso RA, Muda AO, Bersiga A, Rigoli L and Inferrera C. Morphological evidence of neutrophil-tumor cell phagocytosis (cannibalism) in human gastric adenocarcinomas. Ultrastruct Pathol 2002; 26: 315-21.

[4] Lim JJ, Grinstein S and Roth Z. Diversity and versatility of phagocytosis: roles in innate immunity, tissue remodeling, and homeostasis. Front Cell Infect Microbiol 2017; 7: 191.

[5] Uei Y, Watanabe Y, Hirota T, Yamamoto H and Watanabe H. Cytologic diagnosis of breast carcinoma with nipple discharge: special significance of the spherical cell cluster. Acta Cytol 1980; 24: 522-8.

[6] Hong IS. The exfoliative cytology of endometrial stromal sarcoma in peritoneal fluid. Acta Cytol 1981; 25: 277-81.

[7] Masukawa T and Friedrich EG Jr. Cytopathology of Paget's disease of the vulva. Diagnostic abrasive cytology. Acta Cytol 1978; 22: 476-8.

[8] Frable WJ, Bonfiglio TA, Kaminsky DB and Murphy WM. Diagnostic cytology seminar. Acta Cytol 1980; 24: 90-136.

[9] Towers KJ and Melamed MR. Absence of prognostic features in the cytology of effusions due to mammary cancer. Acta Cytol 1979; 23: 30-4.

[10] Craig ID, Desrosiers P and Lefcoe MS. Giant-cell carcinoma of the lung. A cytologic study. Acta Cytol 1983; 27: 293-8.

[11] DeSimone PA, East R and Powell RD Jr. Phagocytic tumor cell activity in oat cell carcinoma of the lung. Hum Pathol 1980; 11 Suppl: 535-9.

[12] Lozupone F and Fais S. Cancer cell cannibalism: a primeval option to survive. Curr Mol Med 2015; 15: 836-41.

[13] Fais S and Fauvarque MO. TM9 and cannibalism: how to learn more about cancer by studying amoebae and invertebrates. Trends Mol Med 2012; 18: 4-5.

[14] Lugini L, Matarrese P, Tinari A, Lozupone F, Federici C, Iessi E, Gentile M, Luciani F, Parmiani G, Rivoltini L, Malorni W and Fais S. Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. Cancer Res 2006; 66: 3629-38.

[15] Matarrese P, Ciarlo L, Tinari A, Piacentini M and Malorni W. Xeno-cannibalism as an exacerbation of self-cannibalism: a possible fruitful survival strategy for cancer cells. Curr Pharm Des 2008; 14: 245-52.

[16] Malorni W, Matarrese P, Tinari A, Farrace MG and Piacentini M. Xeno-cannibalism: a survival "escamotage". Autophagy 2007; 3: 75-7.

[17] Overholtzer M, Mailleux AA, Mouneimne G, Normand G, Schnitt SJ, King RW, Cibas ES and Brugge JS. A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. Cell 2007; 131: 966-79.
[18] Yang YQ and Li JC. Progress of research in cell-in-cell phenomena. Anat Rec (Hoboken) 2012; 295: 372-7.
[19] Ferreira FC, Soares MJ, Carvalho S, Borralho L, Vicente G, Branco S, Correia J and Peleteiro MC. Four cases of cell cannibalism in highly malignant feline and canine tumors. Diagn Pathol 2015; 10: 199.
[20] Lugini L, Lozupone F, Matarrese P, Funaro C, Luciani F, Malorni W, Rivoltini L, Castelli C, Tinari A, Piris A, Parmiani G and Fais S. Potent phagocytic activity discriminates metastatic and primary human malignant melanomas: a key role of ezrin. Lab Invest 2003; 83: 1555-67.
[21] Monteagudo C, Jordá E, Carda C, Illueca C, Peydró A and Llombart-Bosch A. Erythrophagocytic tumor cells in melanoma and squamous cell carcinoma of the skin. Histopathology 1997; 31: 367-373.
[22] Gupta K and Dey P. Cell cannibalism: diagnostic marker of malignancy. Diagn Cytopathol 2003; 28: 86-7.
[23] Lewis CE and Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006; 66: 605-12.
[24] Fiorentini C, Falzano L, Fabbri A, Stringaro A, Logozzi M, Travaglione S, Contamin S, Arancia G, Malorni W and Fais S. Activation of rho GTPases by cytotoxic necrotizing factor 1 induces macropinocytosis and scavenging activity in epithelial cells. Mol Biol Cell 2001; 12: 2061-73.