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Early View

Research letter

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Circulating fibrocytes as a new tool to predict lung cancer progression after surgery?

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To the Editor:

Lung cancer remains the leading cause of cancer death. In 2008, 2,206,771 new cases and 1,796,144 deaths were reported worldwide [1]. At diagnosis, approximately one third of the patients have a potentially resectable tumor that is confined to the chest without clinical evidence of mediastinal lymphadenopathy (clinical stages I and II). Nevertheless, even after complete surgical resection, the overall survival in such patients with early-stage disease remains disappointing. The 5-year survival rate even for patients with stage IA or IB non-small cell lung cancer (NSCLC) is 73% and 56%, respectively [2]. Besides, the 5-year risks of local or regional recurrence after surgery alone for non-small cell lung cancer for stages IA, IB, IIA, IIB, and IIIA are also high (16%, 23%, 37%, 39%, and 30%, respectively [3]). The 5-year survival of small cell lung cancer (SCLC) patients with early-stage resected disease (T1,2 N0M0) is roughly similar, around 50% [4]. Relapse of the disease at distant sites, despite complete removal of all macroscopic lesions, is the main cause of treatment failure [5–8]. The presence of micro-metastatic disease at resection is the likely reason for such relapse. However, the mechanisms involved in micro-metastases propagation remains to be clarified.

Fibrocytes, a distinct population of collagen-producing, monocytes-derived cells, are involved in physiological (wound healing) as well as pathological processes such as idiopathic pulmonary fibrosis [9], where circulating fibrocytes are associated with higher mortality [10, 11]. Accumulating evidence suggests that fibrocytes also play a role in the tumor microenvironment. Indeed, it has been shown that circulating fibrocytes could favor the invasion of cancer cells by inducing the influx of Ly-6C$^+$ monocytes via CCL2 secretion, thus contributing to pre-metastatic conditioning [12]. Moreover, in a recent study conducted in lung cancer samples, a significantly increased number of fibrocytes in bevacizumab-treated tumors suggested that fibrocytes could also mediate acquired resistance to bevacizumab [13]. Thus, the role of fibrocytes in lung cancer physiopathology needs to be investigated.

Our aim was to examine whether circulating and peri-bronchial fibrocytes are associated to disease-free survival (DFS) in lung cancer patients.

We conducted, in a local cohort of patients [14], a *post-hoc* analysis of a prospective study among 35 patients who underwent surgical resection for a very early stage lung cancer (stage IA and IB lung cancer, according to the 7th AJCC TNM classification). This clinical trial was sponsored by the University Hospital of Bordeaux. The study was registered at ClinicalTrials.gov under the N° NCT01692444 (*i.e.,* "Fibrochir" study). The study protocol was approved by the local research ethics committee on May 30, 2012, and the French National Agency for Medicines and Health Products Safety on May 22, 2012. All subjects provided written informed consent. We collected patients'

characteristics: age, gender, smoking habits, lung cancer type, TNM classification. Fragments of the tumor and from distal parenchyma were obtained from lung resection material at a distant of at least 2 cm from the tumor. Fibrocytes were quantified as FSP1 and CD45 double-positive cells in the lamina propria of distal bronchi as previously described [14]. Moreover, circulating fibrocytes were harvested the day of the surgery and immediately quantified following blood sampling, from peripheral blood mononuclear cells (PBMCs) separated from the whole blood, and were identified as double positive cells for the surface marker CD45 and the intracellular marker collagen I by flow cytometry [14].

The primary outcome of interest was the Disease Free Survival (DFS) after surgical resection for stage IA and IB lung cancer. Receiver operating characteristic (ROC) curve analysis was performed for circulating fibrocytes concentration and fibrocytes density in distal tissue specimens and in the tumor to predict cancer progression. Results are median and interquartile range (median [IQR_1; IQR_3]). These 2 cut-off values were then used to evaluate the association between DFS and circulating fibrocytes or density of tissular distal fibrocytes.

Among the 35 patients included 46% were males, 30% current smokers and 34% suffered from COPD. Mean age was 64.8 years (±13.2). Histology of resected lung cancer was for 23 (66%) adenocarcinoma, 5 (14%) squamous cell carcinoma and 7 (20%) other histology. Twenty-two (63%), 10 (28%) and 3 (9%) were respectively pT1, pT2 and pT3 according to the 7^{th} TNM classification, all being R0. Follow-up duration and DFS were 1902 [1581; 2316] and 1516 [995; 1780] days respectively. The median percentage of circulating fibrocytes among PBMC was 6.2% [2.1; 11.6].

The median density of peri-bronchial fibrocytes was 9.0 [3.6; 29.4] x 10^6 cells/m^2 and the median density of intra-tumoral fibrocytes was 2.0 [1.5; 3.9] x 10^6 cells/m^2. Among the 35 patients, 35% encountered progression of the disease. Comparison between non-progressors and progressors after follow-up did not show any significant difference in demographic characteristics but revealed a significant difference in circulating fibrocytes percentages (7.4 *versus* 3.5%, respectively, p=0.01). Using ROC analyses to predict progressive status according to circulating fibrocytes, the area under the curve (AUC) was 0.75 (p=0.01) with an optimal cut-off of 6% for a sensitivity of 75% and a specificity of 70% (Figure 1A). We used this cut-off to divide patients in 2 groups. Patient's characteristics were not significantly different between the two groups. By contrast, patients with a percentage of circulating fibrocytes below 6% had a significantly shorter DFS (1107 days *versus* 1626 days, p<0.0001, Figure 1B). Not surprisingly, as circulating fibrocytes percentage is positively correlated with peri-bronchial fibrocytes density (Spearman, p<0.001, rho=0.7)[14], we observed a similar significant relationship for tissue fibrocytes density (p=0.03, Figure 1C and D). Similarly intra-tumoral fibrocytes were also significantly higher among non-progressors (2.3 *versus* 1.6 x 10^6 cells/m^2, p=0.03). Of note, the observed effect seemed to be specific to fibrocytes and not related to

COPD, as a similar proportion of COPD patients was observed in both groups (5 (30%) *versus* 7 (35%), p= 0.46).

Recurrence after surgery remains unacceptably high, even for patients with stage I disease in whom neither nodal nor other metastatic involvement were detected at the time of surgery. This is the first study showing a link between circulating fibrocytes and prognosis in early stage of lung cancer after surgery with a long-term follow-up. Identifying a new biomarker is a major issue to adapt patients' care and/or modulate treatment in order to avoid recurrence. Indeed, early detection and relapse from lung cancer prediction is of major interest for both patients and healthcare professionals to better manage costs, treatment intensity and time spent around medical care. Although some progress has been made on the early prognosis of cancer, further studies are required to identify biomarkers that are suitable for medical use in clinical setting in order to estimate progression. Ultimately, predicting the exact survival period after diagnosis increases the prognostic accuracy and might lead to better informed decision making both for the patient and in terms of physician's efforts.

With regard to the mechanism, current literature indicates a detrimental role for fibrocytes in tumors' microenvironment. However, we observed here that a higher proportion of circulating and intra tumoral fibrocytes was associated with a better prognosis. As fibrocytes are able to remodel the extracellular matrix by collagen secretion or endocytosis [15], or even via extracellular vesicles secretion [16], such micro-environmental tissue remodelling may prevent metastatic cells dissemination. Moreover, recent data pointed out the beneficial role of fibrocytes in lung cancer: by a direct interaction with $CD8^+$ cytotoxic T cells via CD86/CD28 co-stimulation, fibrocytes have been shown to promote $CD8^+$ T cells proliferation and act synergically with PD-L1/PD-1 blockade [17].However, we did not find any association between the number of intra tumoral of CD8 T cells and progression. Fibrocytes could play a role not directly in the microenvironment of the tumor but in micrometastasis genesis. Overall, they could reduce metastatic risk by stimulating anti-tumoral auto-immunity. However, due to some limitations such as the absence of a validation cohort and the small number of patients, further studies are still required to clarify the mechanism linking fibrocytes and lung cancer survival.

In conclusion, circulating fibrocytes could act as an additional tool to identify patients who will suffer from a worse-than-expected prognosis after surgery for early-stage lung cancer, since a lower concentration of circulating fibrocytes was associated with a shorter disease-free survival.

FIGURE LEGEND

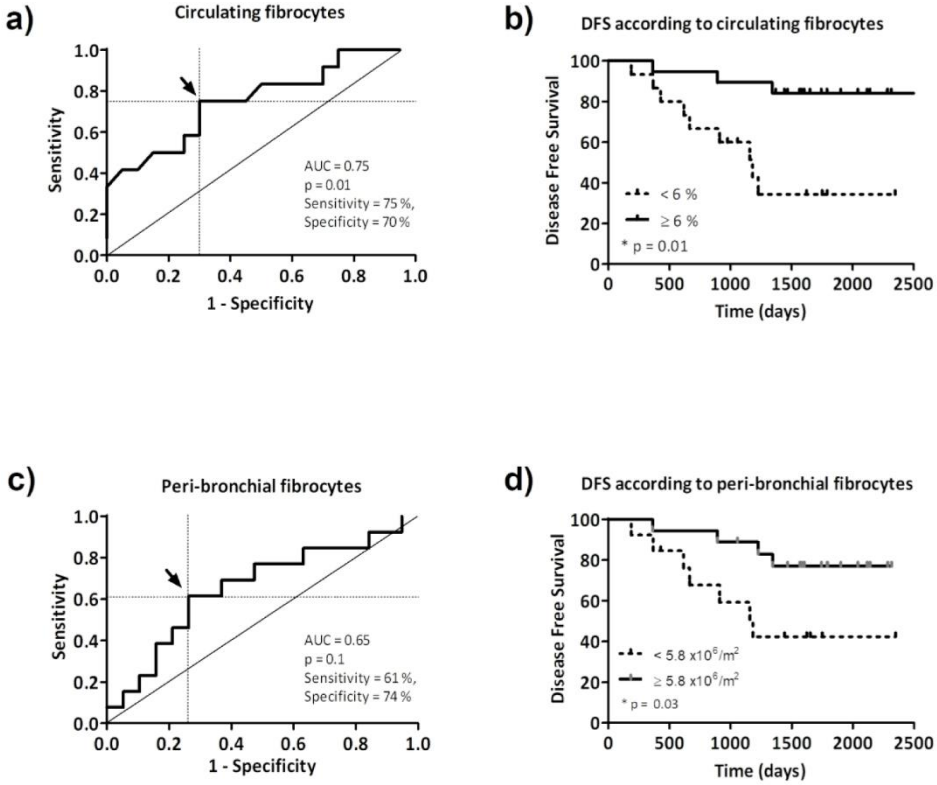

Figure 1: Circulating and peri-bronchial fibrocytes are able to significantly predict longer disease-free survival. ROC, Receiver Operating Curve. PBMC, peripheral blood mononuclear cells

a) ROC analysis for circulating fibrocytes. The arrow represents the optimal circulating fibrocytes threshold (i.e. 6% of PBMC), with a sensitivity of 75% and a specificity of 70%.

b) Kaplan-Meier survival curve according to the threshold of 6% of PBMC for circulating fibrocytes. Survival is significantly different between the two groups (p=0.01).

c) ROC analysis for peri-bronchial fibrocytes. The arrow represents the optimal peri-bronchial fibrocytes threshold (i.e. 5.8 / m^2), with a sensitivity of 61% and a specificity of 74%.

d) Kaplan-Meier survival curve according to the threshold of 5.8 peri-bronchial fibrocytes / m^2. Survival is significantly different between the two groups (p=0.03).

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