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# Non-professional phagocytosis in tissue micro arrays of breast cancer patients

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## 1. Background

Phagocytic clearance of diseased, dying or dead cells is an important mechanism to maintain the natural homeostasis of tissues. Previous studies show that phagocytosis can not only be performed by macrophages or dendritic cells, but also by so-called non-professional phagocytes. Both certain healthy and cancerous cell types act as non-professional phagocytes as they are able to engulf their neighbouring cells under specific circumstances forming cell-in-cell structures (CICs). CIC formation emerges as an important prognostic marker for several cancer types. We studied if breast carcinoma cell lines have the ability to perform non-professional phagocytosis. Furthermore, frequency and prognostic significance of CICs in breast cancer tissue micro arrays are studied.

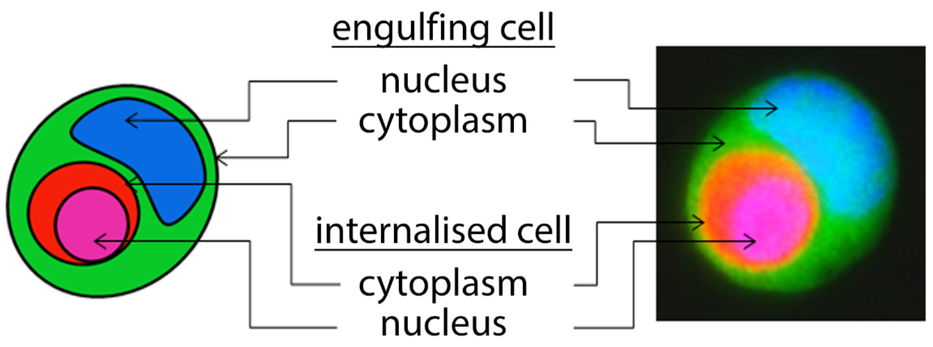


**Fig.1: Characteristics of cell-in-cell structures. The internalized cell is round and completely surrounded by the engulfing cell's cytoplasm whilst the engulfing cell's nucleus notched at the side facing the internalized cell.**

## 2. Methods

Healthy human fibroblasts (SBLF-9) and two breast cancer cell lines (MDA-MB-231, MCF-7) were stained red and treated with hyperthermia (56°C) for 1h. Afterwards they were incubated with a homotypic cell layer for 4h, fixed, blocked and stained immunohistochemically the following day.
147 breast cancer patients with a pTNM stage from 1mic to 2 were comprised in the study. 601 samples originating from the centre of the tumour, tumour infiltration, cancerous tissue close to or far from the lymphatic node, normal lymphatic nodes or lymphatic metastasis were included. The samples were transformed into tissue micro arrays (TMAs) and immunohistochemically stained for E-cadherin to visualize cell membranes. CICs were counted using Biomas image processing software.

## 3. In vitro results

On average, 1.9% of living MDA-MB-231 and 1.5% of living MCF-7 formed CICs after the incubation time of 4h. Therefore, breast cancer cell lines are able to perform non-professional phagocytosis with a slightly higher CIC rate than human fibroblasts, which only formed 0.6% CICs on average.

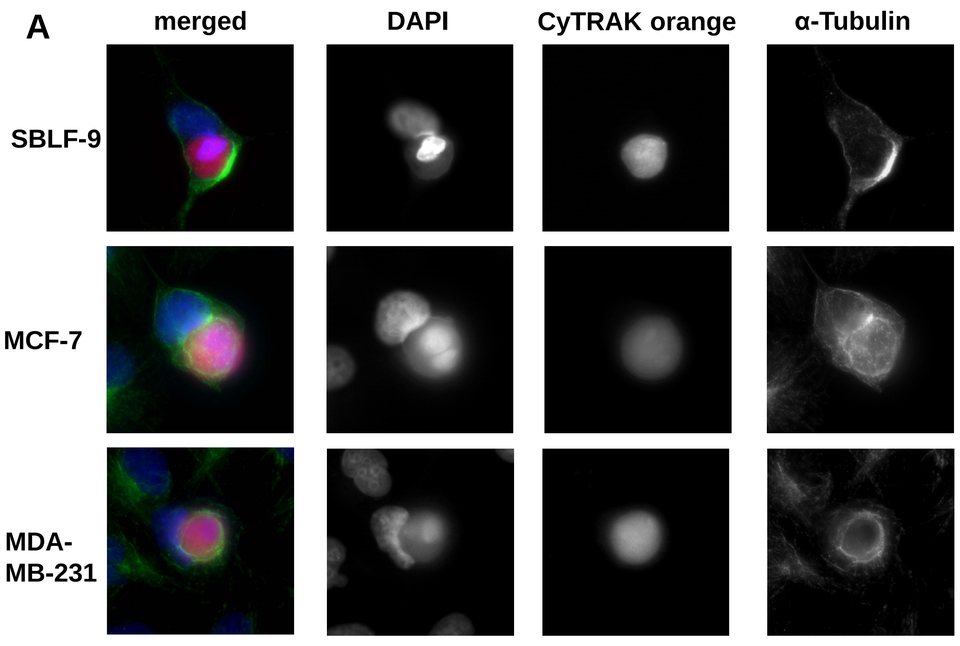


**Fig.2A: Cell-in-cell structures formed by homotypic non-professional phagocytosis. The blue signal represents DAPI; the red signal represents CyTRAK orange (dye to label necrotic cells); the green signal represents α-Tubulin.**
**Fig.2B: Comparison of percentages of CIC formation between SBLF-9 and the breast cancer cell lines.**

## 4. CICs in tissue micro arrays

83% of the included patients were either staged pTNM 1c or pTNM 1b; the remaining 17% of patients had smaller (pTNM 1mic, 1a) or larger (pTNM 2) tumours.
CICs were found in 21.8% of all samples, mostly in central tumour TMAs and tumour infiltration zone TMAs. The rate ranged from 5.9 x $10^{-6}$ to 556.5 CICs per $mm^2$. Proportionally, more CICs were found in central tumour and tumour infiltration TMAs.
Referring to the fact that one to a maximum of six samples originated from the same patient, CIC formation was observed in 61% of the patients in at least 1 sample. As Figure 3c indicates, more than 2 positive samples per patient were hardly found.

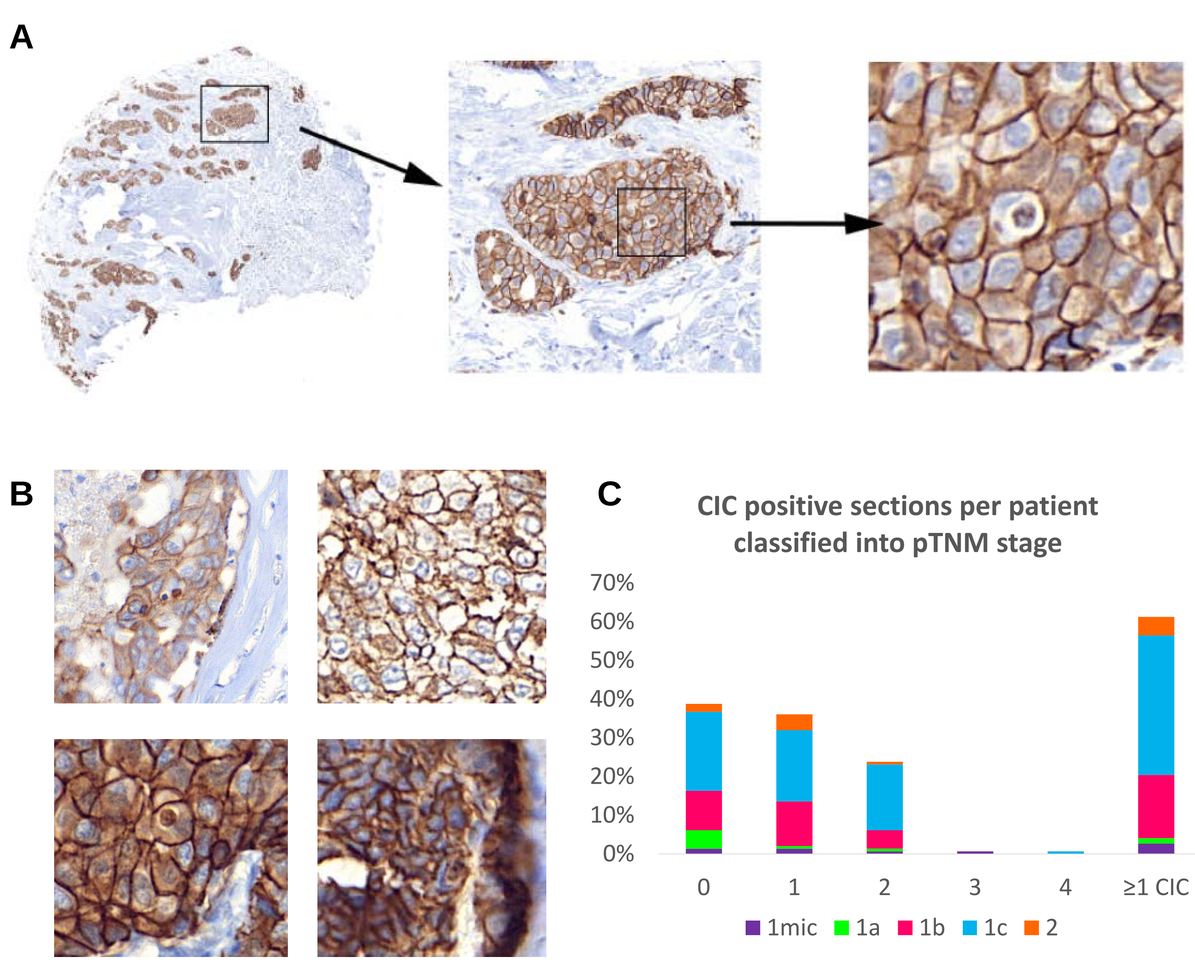


**Fig.3A: TMA with magnified sections to indicate analysis procedure. Fig. 3B: TMA sections with CICs. Fig.3C: Percentage of CIC-positive sections per patient. Subgroups represent the pTNM stage of the patient.**

Patients with CIC formation present in their TMAs have a longer recurrence free and overall survival. However, metastases evolve earlier if CICs are present in the primary tumour.

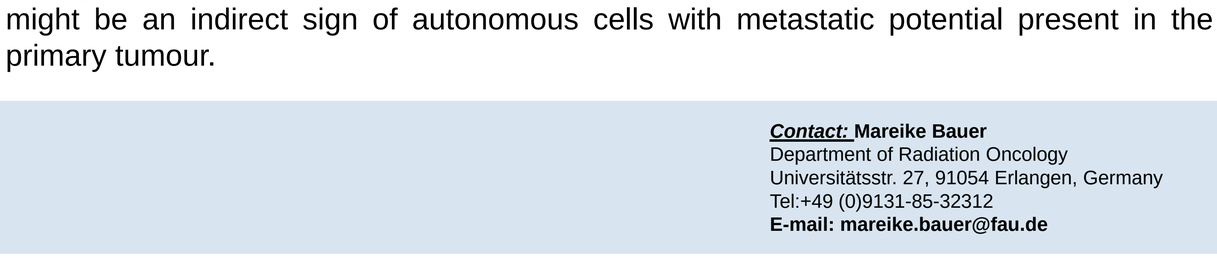


**Fig.4: Kaplan Meier plots of recurrence free survival, metastasis free survival and overall survival depending on the presence of CICs in the primary tumour. Green represents CIC positivity; blue represents CIC negativity.**

## 5. Conclusion

Breast cancer cells are indeed able to perform non-professional phagocytosis, both in vitro and in vivo.

Only necrotic cells were phagocytosed by viable homotypic cells, whereas they didn't engulf their viable neighbouring cells. Therefore, the in vitro results suggest that breast cancer cells use non-professional phagocytosis to dispose cells outside of the united cell structure.

Taking into account that CIC formation in vivo increases the risk of metastases, CICs might be an indirect sign of autonomous cells with metastatic potential present in the primary tumour.

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