Cell fusion model to investigate a potential origin of circulating tumor cells

Sabine Klatt[1], Theresa Wießner-Kroh[1], Gudrun Marquardt[1], Miriam Faxel[1], Stefan Rubner[1#], Ioannis Papasotiriou[2#]

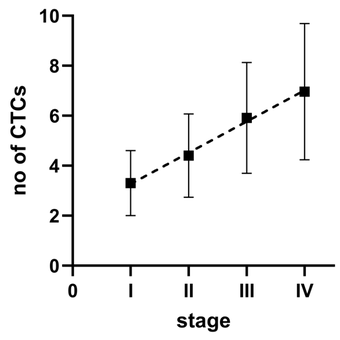
[1] Research Genetic Cancer Centre Central Europe GmbH, Weinbergweg 22, D-06120 Halle (Saale), Germany
[2] Research Genetic Cancer Centre International GmbH, Baarerstrasse 95, CH-6300 Zug, Switzerland
[#] These authors contributed equally

Background

Cell fusion is a natural process in which the membranes of two or more cells fuse to one hybrid cell containing features of the original cells. In pathological context, this process is implicated to play a key role in cancer initiation, progression and the process of metastasis since hybrid cells were verified in human cancers. Especially, the cell fusion between cancer cells and immune cells of the tumor microenvironment could lead to hybrid cells with unique properties favoring cancer progression and escape immune response with potential impact on drug and apoptosis resistance.[1,2] Interestingly, circulating tumor cells (CTCs) might originate from this transformation gaining new features to detach from the primary tumor, and metastasize to distant organs. However, the process of cancer-associated cell fusion and its consequences is poorly understood because of the lack of suitable study models.

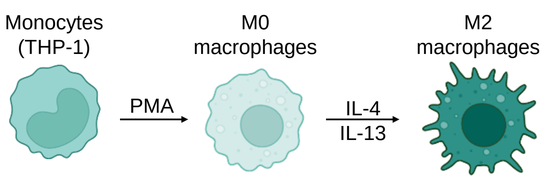
CTCs

A comprehensive study analyzed more than 14.000 patient samples revealing a positive correlation of CTC number and cancer stage in line with presence of metastasis at later stages and poor prognosis.[3]

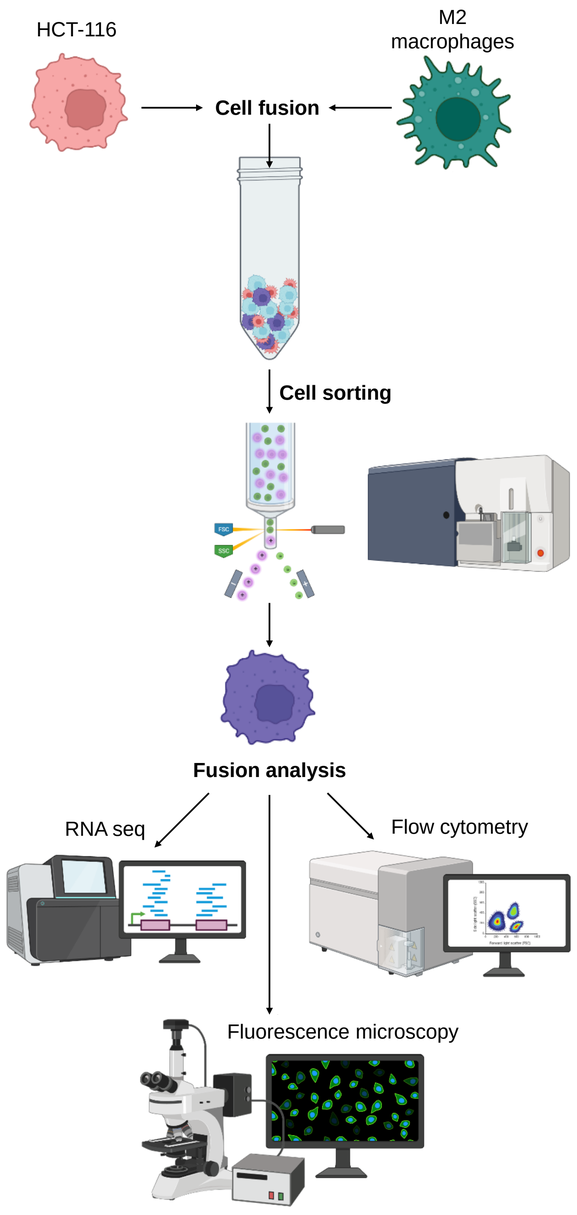


Cell fusion model

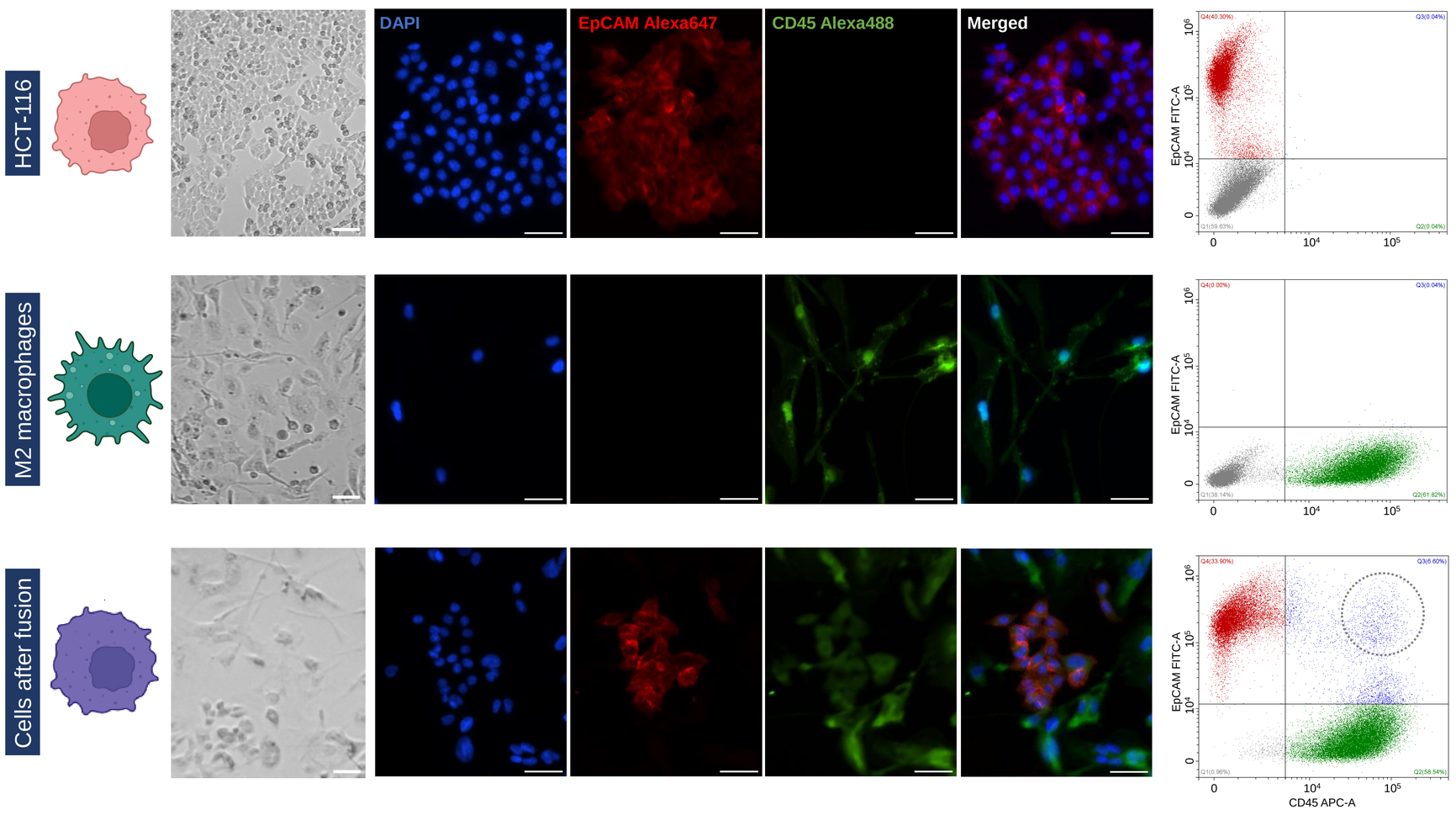
The monocytic cell model THP-1 was differentiated to M0 and M2 macrophages by Phorbol 12-myristate 13-acetate (PMA) or combined interleukin 4/13 (IL-4/IL-13) treatment, respectively.



For the cell fusion approach, the colon cancer cell line HCT-116 and M2 macrophages derived from THP-1 cells were fused by a PEG-based fusion protocol. The resulting heterogenous cell population were sorted for EpCAM and CD45, exclusively expressed on either HCT-116 or THP-1 cells. Double positive cells were further investigated on RNA and protein level.

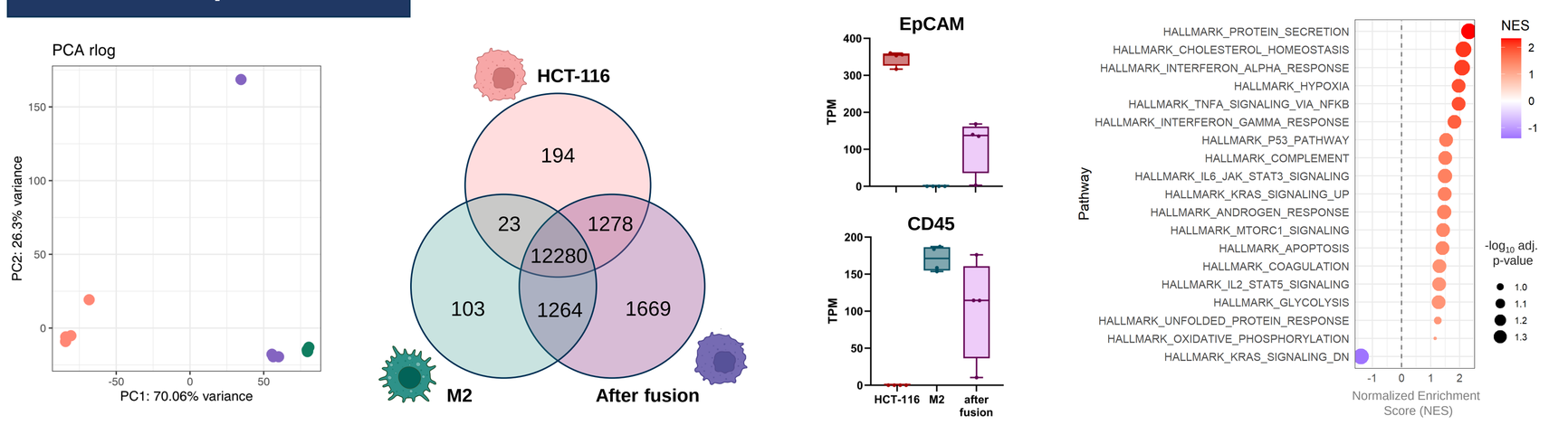


Phenotypical cell features



Characterization of HCT-116, M2 macrophages and double-positive cells (after fusion) was performed by brightfield (left panel) as well as fluorescent microscopy (middle panel) and flow cytometry (right panel). Co-localization of the specific cell surface marker EpCAM and CD45 were demonstrated by fluorescence microscopy (Scale bar: 50 µm). Moreover, flow cytometry analysis identified a population of double-positive cells which are circled within the intensity dot plot.

Transcriptomics



A unique RNA expression profile was discovered upon analysis of whole transcriptome sequencing data for double-positive cells (after fusion) shown by PCA plot and Venn diagram of expressed genes. Among others, expression of both cell type-specific selection marker were detected in these cell population. The gene set enrichment analysis revealed an activation of immune-related pathways for double-positive cells in comparison to HCT-116 (n=4).

Conclusion

- *In vitro* cell fusion model could be established.
- Cell fusion is indicated by fluorescence microscopy, flow cytometry and transcriptional data.
- Double-positive cells harbor features of both parental cell types.

Outlook

- Transcriptional comparison of double-positive cells with CTCs originated from colorectal cancer.
- Cultivation of double positive cells to proof on RNA and protein level if newly achieved features are permanently expressed.
- Analysis of migratory potential of fused cells.

References:
1. Manjunath *et al.* (2020) Tumor-Cell-Macrophage Fusion Cells as Liquid Biomarkers and Tumor Enhancers in Cancer, Int J Mol Sci, doi: 10.3390/ijms21051872
2. Zhang *et al.* (2019) Fusion of macrophages promotes breast cancer cell proliferation, migration and invasion through activating epithelial-mesenchymal transition and Wnt-ß-catenin signaling pathway, ABB doi.org/10.1016/j.abb.2019.108137
3. Paz *et al.* (2023) Correlation of Circulating Tumor Cells with Cancer Stage, Poster presentation ESMO Congress Barcelona

Disclosure of Potential Conflicts of Interest:
None of the authors of the above study has declared any conflict of interest.

Contact details:
office@rgcc-centraleurope.com