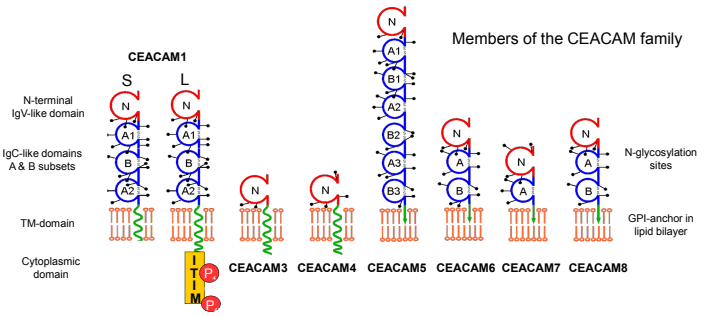
Functional role of CEACAM1 in the lymphogenic metastasis of the prostate carcinoma

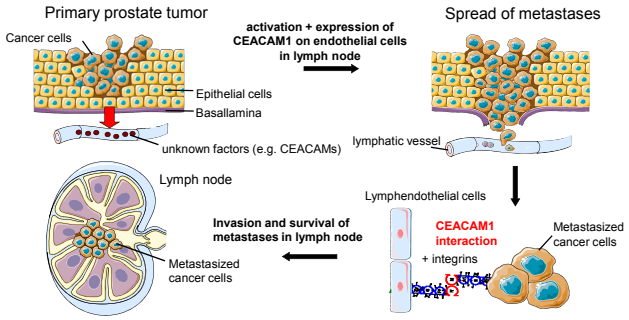
E. Nilewski[1], A. Görgens[2], H.T. Muturi[1], B. Giebel[2], S. Ergün[3], D. Tilki[4] and B.B. Singer[1]

[1]Anatomy, University Hospital Essen; [2]Transfusion Medicine, University Hospital Essen; [3]Anatomy and Cell Biology, University Hospital Würzburg, [4]Urological Clinic and Polyclinic, University Hospital Munich

Introduction

Prostate carcinoma is the most common cancer type in men above 50 years of age. Mostly, the cancer grows very slowly but often metastasizes via the lymphatic system. Essential for the metastatic process are adhesion molecules. CEACAM1, a member of the carcinoembryonic antigen (CEA) family, is a homophilic/heterophilic cell adhesion molecule with angiogenic and lymphangiogenic properties. It exists in short and long isoforms (CEACAM1-S and -L) and is expressed on most epithelial cells, angiogenically activated endothelial cells and leukocytes. CEACAM1, but no other CEACAMs are expressed in normal prostate tissue, and is down-regulated in prostate carcinoma (PIN, Gleason grade 4, 5). Interestingly, CEACAM1 is absent in quiesent endothelial cells, but appears in tumor-associated blood and lymphendothelial cells. Our previous results (Immunohistochemistry) showed an expression of CEACAM1 in sinusoidal endothelial cells of lymph nodes in patients with prostate carcinoma as well as in tumor cell clusters found within lymphatic vessels. Based on these findings we hypothesized an important role of CEACAM1 in lymphangiogenesis and lymphatic metastasis of prostate cancer. To test this, we established a mouse and human *in vitro* model. For the mouse system, we used different prostate cancer cell lines (AD-Ca) and the endothelial cell line bEnd.3. For the human model, we analyzed the prostate cancer cell line PC-3 and the lymphendothelial cell line AS-M.5.





Objectives

- Characterization of the CEACAM expression pattern in lymphendothelial and prostate carcinoma cell lines
- Analyses of homophilic/heterophilic CEACAM interaction between tumor and lymphendothelial cells in murine and human *in vitro* model

Results murine model

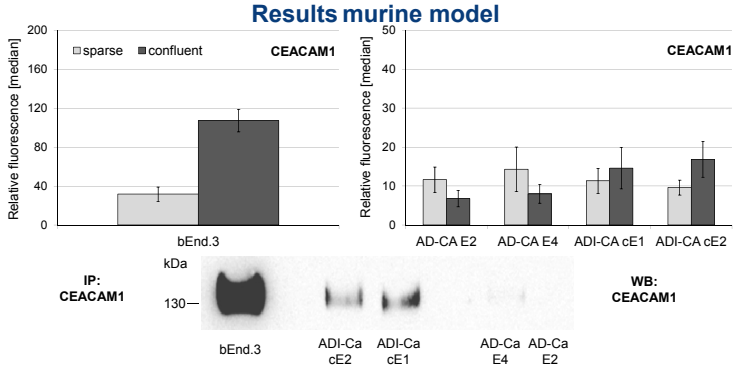


Figure 1: Confluent endothelial cells bEnd.3 express more CEACAM1 than proliferating cells. The murine prostate carcinoma cell lines (AD-Ca E2/E4, ADI-Ca cE1/cE2) express barely CEACAM1. Flow cytometry and Western Blot of confluent and proliferating cells was performed ultilizing mAb anti-mCC1.

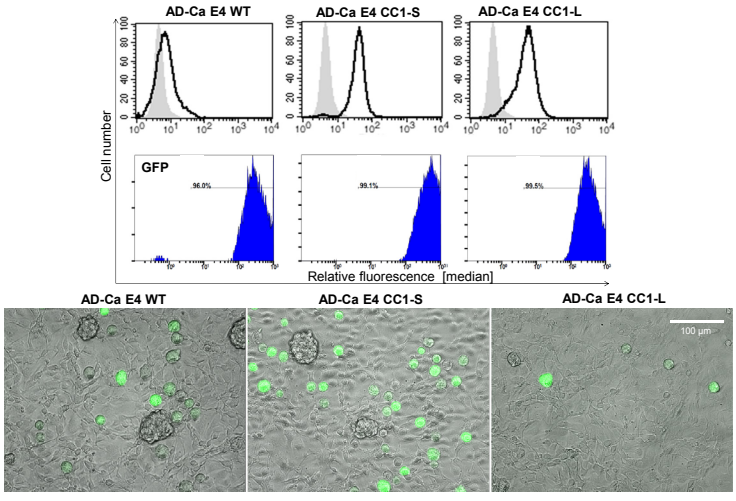


Figure 2: Prostate carcinoma cells over-expressing CEACAM1-S show the highest capacity to adhere to the confluent endothelial cell monolayer bEnd.3 compared with WT and CEACAM1-L transfected cells. Transfection efficiency of cells with pLXSN-CEACAM1-S/L and GFP was confirmed by flow cytometry. For the interaction studies a confluent monolayer of bEnd.3 was incubated with different GFP-labeled AD-Ca transfectants. The CEACAM1 interaction mediated was monitored by fluorescence microscopy.

Results human model

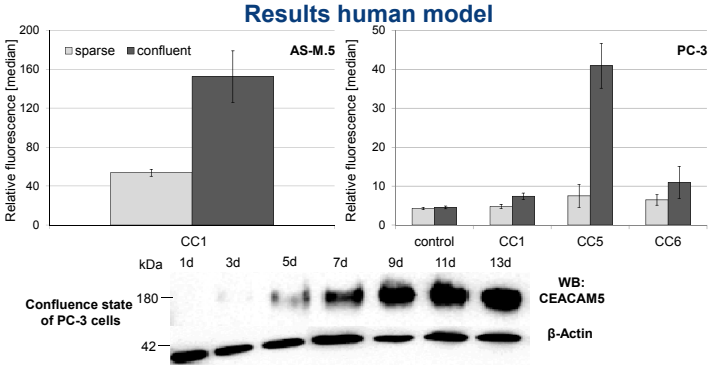


Figure 3: Confluent endothelial cells AS-M.5 express more CEACAM1 than proliferating cells. Confluent prostate carcinoma PC-3 cells express no CEACAM1, barely CEACAM6 and a significant amount of CEACAM5. Flow cytometry and Western Blot analysis of confluent and proliferating cells was performed ultilizing mAb B3-17 (anti-hCC1), mAb 5C8C4 (anti-hCC5) and mAb 1H7-4B (anti-hCC6), respectively.

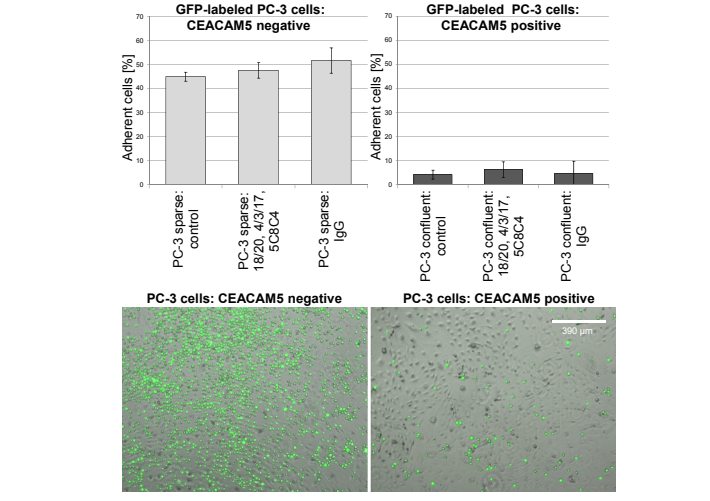


Figure 4: CEACAM5 negative PC-3 cells show a higher adherence to the confluent endothelial monolayer AS.M-5 than CEACAM5 positive PC-3. CEACAM binding mAbs do not alter the amount of PC-3 cells adhered to AS-M.5. Confluent AS-M.5 monolayers were incubated with GFP-labeled PC-3 cells harvested from different growth stages. In some cases mAbs anti-CEACAM1/5 or IgG were used to analyze the potential impact of CEACAM1 and CEACAM5 in this model. The tumor-endothelial cell interactions were monitored by a fluorescence reader and fluorescence microscopy.

Conclusion

- Confluent endothelial cells express more CEACAM1 than proliferating cells.
- Murine prostate carcinoma cell lines express low level of CEACAM1, whereas the human prostate carcinoma cell line PC3 does express no CEACAM1, barely CEACAM6, but a significant amount of CEACAM5. Other human prostate cancer cell lines analyzed do not express any CEACAMs (e.g. DU-145, LNCaP).
- In mouse, prostate carcinoma cells over-expressing CEACAM1-S, but not CEACAM1-L show an increased adhesion to confluent lymphendothelial cells.
- In human, PC-3 cells expressing CEACAM5 show a decreased adherence to confluent lymphendothelial cells compared to CEACAM5 negative cells.

Hence, the CEACAM1-CEACAM1 interaction supports, while the CEACAM1-CEACAM5 interaction prohibits lymphogenic metastasis of prostate carcinoma

Supported by DFG (ER276/9-1)