CD44+/CD74+ CTCs 与肿瘤脑转移密切相关

肿瘤脑转移是非小细胞肺癌 (NSCLC)、乳腺癌及恶性黑色素瘤较常见的转移途径，占比高达 40%，对肿瘤患者造成很大危害，近年来在全球范围内有升高趋势。因此，如何有效预测肿瘤脑转移对进一步指导肿瘤患者精准治疗具有非常重要的临床意义。最近，国际著名的德国 CTC 专家 Klaus Pantel 和 Harriet Wikman 教授率领的团队，针对上述三种肿瘤脑转移患者的 CTC 进行了研究，结果揭示，表达 CD44 及 CD74 的 CTCs 广泛存在于肿瘤脑转移患者体内，可作为判断肿瘤脑转移的有效指标，相关文章刚刚得到发表[1]。 赛特生物林平博士应邀作为文章的审稿人之一，针对本文及今后与肿瘤脑转移相关的 CTC 研究向作者提出了一系列积极建议。

International Journal of Molecular Sciences

MDPI

Article

CD74 and CD44 Expression on CTCs in Cancer Patients with Brain Metastasis

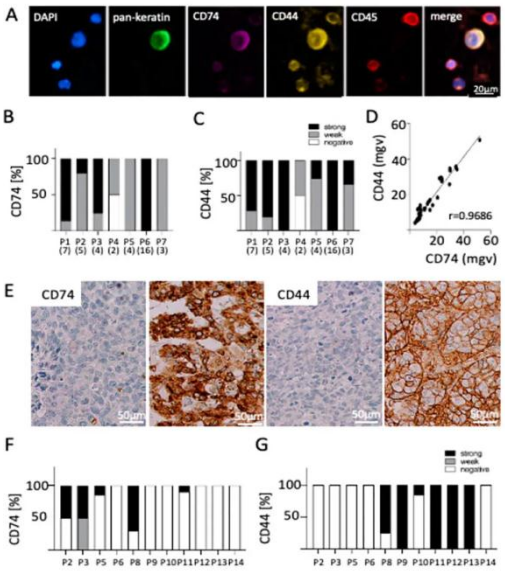
Desiree Loreth [1], Moritz Schuette [2], Jenny Zinke [3,4,5], Malte Mohme [6], Andras Piffko [6], Svenja Schneegans [1], Julia Stadler [7], Melanie Janning [8,9,10], Sonja Loges [8,9,10], Simon A. Joosse [1], Katrin Lamszus [6], Manfred Westphal [6], Volkmar Müller [11], Markus Glatzel [12], Jakob Matschke [12], Christoffer Gebhardt [7], Stefan W. Schneider [7], Iwona Belczacka [13], Beate Volkmer [14], Rüdiger Greinert [14], Marie-Laure Yaspo [15], Patrick N. Harter [3,4,5], Klaus Pantel [1] and Harriet Wikman [1,*]

1 Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; d.loreth@uke.de (D.L.); s.schneegans@uke.de (S.S.); s.joosse@uke.de (S.A.J.); pantel@uke.de (K.P.)
2 Alacris Theranostics GmbH, Max-Planck-Straße 3, 12489 Berlin, Germany; m.schuette@alacris.de
3 Institute of Neurology (Edinger Institute), Goethe University, 60528 Frankfurt am Main, Germany; jenny.zinke@gmx.net (J.Z.); patrick.harter@kgu.de (P.N.H.)
4 German Cancer Consortium (DKTK), Partner Site Frankfurt/Mainz, 60528 Frankfurt am Main, Germany
5 German Cancer Research Center (DKFZ), Heidelberg, Germany; Frankfurt Cancer Institute (FCI), 60528 Frankfurt am Main, Germany
6 Department of Neurosurgery, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; m.mohme@uke.de (M.M.); a.piffko@uke.de (A.P.); lamszus@uke.uni-hamburg.de (K.L.); westpha3@uke.de (M.W.)
7 Department of Dermatology and Venerology, University Hospital Hamburg-Eppendorf, 20246 Hamburg, Germany; j.stadler@uke.de (J.S.); ch.gebhardt@uke.de (C.G.); st.schneider@uke.de (S.W.S.)
8 Division of Personalized Medical Oncology (A420), German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany; melanie.janning@medma.uni-heidelberg.de (M.J.); s.loges@dkfz.de (S.L.)
9 Department of Personalized Oncology, University Hospital Mannheim, Medical Faculty Mannheim, University of Heidelberg, 68167 Mannheim, Germany
10 Department of Oncology, Hematology and Bone Marrow Transplantation with section Pneumology, Hubertus Wald Comprehensive Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany
11 Department of Gynecology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; v.mueller@uke.de
12 Department of Neuropathology, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany; m.glatzel@uke.de (M.G.); matschke@uke.de (J.M.)
13 Otto Warburg Laboratory Gene Regulation and Systems Biology of Cancer, Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany; i.belczacka@gmail.com (I.B.); yaspo@molgen.mpg.de (M.-L.Y.)
14 Association of Dermatological Prevention, Germany & Centre of Dermatology, Elbe Clinics, 21614 Bustehude, Germany; beate.volkmer@bekliniken.de (B.V.); ruediger.greinert@bekliniken.de (R.G.)
* Correspondence: h.wikman@uke.de; Tel.: +49-(407)-510-51913

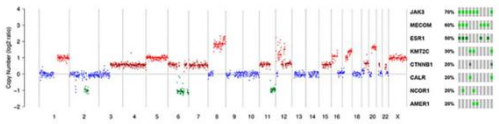
Abstract: Up to 40% of advance lung, melanoma and breast cancer patients suffer from brain metastases (BM) with increasing incidence. Here, we assessed whether circulating tumor cells (CTCs) in peripheral blood can serve as a disease surrogate, focusing on CD44 and CD74 expression as prognostic markers for BM. We show that a size-based microfluidic approach in combination with a semi-automated cell recognition system are well suited for CTC detection in BM patients and allow further characterization of tumor cells potentially derived from BM. CTCs were found in 50% (7/14) of breast cancer, 50% (9/18) of non-small cell lung cancer (NSCLC) and 36% (4/11) of melanoma patients. The next-generation sequencing (NGS) analysis of nine single CTCs from one breast cancer patient revealed three different CNV profile groups as well as a resistance causing ERS1 mutation. CD44 and CD74 were expressed on most CTCs and their expression was strongly correlated, whereas matched breast cancer BM tissues were much less frequently expressing CD44 and CD74 (negative in 46% and 54%, respectively). Thus, plasticity of CD44 and CD74 expression during trafficking of CTCs in the circulation might be the result of adaptation strategies.

Keywords: brain metastasis; circulating tumor cells; breast cancer; NSCLC; melanoma

CD74 表达于免疫细胞及肿瘤细胞表面，其在免疫监视、细胞迁移及信号传导等方面发挥着重要作用。CD44 是重要的干细胞标志物，在肿瘤形成及肿瘤转移过程中的特殊功能已有很多报道。表达在原发灶肿瘤细胞表面的 CD44 和 CD74 相互作用，与 NSCLC、乳腺癌脑转移相关[2]，但两者在 CTC 上的表达与脑转移如何关联以往没有任何报道。



乳腺癌CTC 单细胞测序



本文要点：

- 85.7% 乳腺癌脑转移患者及 71.4% NSCLC 脑转移患者体内均能检测出 CD44、CD74 双阳 CTCs，CD74 的表达与 CD44 高度关联

- CD44、CD74 在 CTC 和原发灶肿瘤细胞上的表达完全不同，大多数含有 CD44、CD74 双阳 CTCs 的患者，其原发灶肿瘤细胞仅表达单一的 CD44 或 CD74
- 单细胞 NGS 测序再次证明 8 号染色体在 CTC 中高度扩增，同时也证实乳腺癌脑转移 CD44、CD74 双阳 CTCs 中存在雌激素受体 (ERS1) 基因突变

CD44、CD74 在 CTC 和原发灶肿瘤细胞上的表达完全不同，这与我们已发表的使用赛特 SE-i•FISH 技术可在大部分病理组织 $hHER2^-$ 胃癌患者或病理组织 $hPD\text{-}L1^-$ NSCLC 患者体内有效检测出 $HER2^+$ 或 $PD\text{-}L1^+$CTC 、CTEC 的结果相一致[3,4]。这意味着 CTC、CTEC 进入血液循环后，出于生长环境改变而适者生存的需要(adaptation)，其细胞表面标志物蛋白能够“可塑性”地动态演变成与原发灶肿瘤细胞完全不同的表型[1]，进而造成 CTC、CTEC 具有与原发灶肿瘤对化疗药物完全不同的敏感性特征[5, 6]。相较于临床以往应用常规病理手段检测病理组织中的 PD-L1 或 HER2 表达以确定适于开展相关治疗的肿瘤患者，动态检测具有转移能力的 CTC 和 CTEC 上的 PD-L1、HER2 等瘤标表达，为肿瘤患者开展靶向或免疫治疗提供了更有效的方法，具有非常重要的现实意义。对于肿瘤的治疗，不应该只局限于原发灶肿瘤，更要以清除在肿瘤转移过程中起重要作用的 CTC、CTEC 为目的，这一点在国外早已报道并引起广泛关注[6]。

相对于单纯的 CTC 计数，本文再次证明在 CTC 上检测各种瘤标蛋白表达的必要性，同时也为“无创性检测 CTC 可以预测肿瘤患者脑转移”提供了有力依据。但本实验因方法的局限性，丢失了所有具有重要临床意义的小细胞 CTCs[7-9]，而且也没有能够特异性地区分 CTC ($CD31^-$) 和 CTEC ($CD31^+$) 如何表达 CD44、CD74 及与肿瘤脑转移的相关性[10]。所有这些都是今后在大规模样本上进一步深入开展相关研究需要密切关注的重要指标。

相关文献：

1. Loreth et al. 2021 CD74 and CD44 Expression on CTCs in Cancer Patients with Brain Metastasis. Int J Mol Sci 22:6993.
2. Boral et al. 2017 Molecular characterization of breast cancer CTCs associated with brain metastasis. Nat Commun 8:196.
3. Li et al. 2018 Evolutionary Expression of HER2 Conferred by Chromosome Aneuploidy on Circulating Gastric Cancer Cells Contributes to Developing Targeted and Chemotherapeutic Resistance. Clin Cancer Res 24:5261.

4. Zhang et al. 2020 PD-L1(+) aneuploid circulating tumor endothelial cells (CTECs) exhibitresistance to the checkpoint blockade immunotherapy in advanced NSCLC patients. Cancer Lett 469:355.
5. Pantel et al. 2004 Dissecting the metastatic cascade. NatRev Cancer 4:448.
6. Pierga et al. 2008 Circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer in a phase II randomized trial.Clin Cancer Res 14:7004.
7. Chen et al. 2021 Dysregulated KRAS gene-signaling axis and abnormal chromatin remodeling drive therapeutic resistance inheterogeneous-sized circulating tumor cells in gastric cancer patients. Cancer Lett 517:78.
8. Hong et al. 2021 Small Cell Size Circulating Aneuploid Cellsas a Biomarker of Prognosis in Resectable Non-Small Cell Lung Cancer. Front Oncol 11:590952.
9. Wang et al. 2018 Quantified postsurgical small cell size CTCs and EpCAM(+) circulating tumor stem cells with cytogenetic abnormalitiesin hepatocellular carcinoma patients determine cancer relapse. Cancer Lett 412:99.
10. Lin 2020 Aneuploid Circulating Tumor-Derived Endothelial Cell (CTEC): A Novel Versatile Player in Tumor Neovascularization and Cancer Metastasis. Cells 9:1539.