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Identification of circulating tumor cells: a prognostic marker in squamous cell carcinoma of the head and neck?

Kris R. Jatana, M.D.,
Assistant Professor, Department of Otolaryngology—Head and Neck Surgery, The Ohio State University and Nationwide Children's Hospital, 555 South 18th Street, Suite 2A, Columbus, Ohio 43205, (614) 722-6040, Phone, (614) 722-6609, Fax

Jas C. Lang, Ph.D., and
Associate Professor, Department of Otolaryngology-Head and Neck Surgery, The Ohio State University, Tzagournis Medical Research Facility, 420 West 12th Avenue, Room #444, Columbus, OH 43210, (614) 292-6026, Phone, (614) 293-7292, Fax

Jeffrey J. Chalmers, Ph.D.
Professor, William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 W. 19th Avenue, Columbus, Ohio 43210, (614) 292-2727, Phone, (614) 292-3769, Fax

Kris R. Jatana: Kris.Jatana@osumc.edu; Jas C. Lang: Lang.8@osu.edu; Jeffrey J. Chalmers: Chalmers@chbmeng.ohio-state.edu

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In 2010, according to the NCI, 49,260 new cases of oral cavity, pharyngeal, and laryngeal cancer were estimated to occur in the United States; approximately 11,480 deaths were attributed to these cases. More than 95% of these cases are squamous cell carcinomas (1, 2). For all stages combined, the 5-year survival rate is approximately 50% (3), and this rate has not changed significantly in the last several decades. Treatment failure in patients with squamous cell carcinoma of the head and neck (SCCHN) can include local recurrence, regional recurrence (cervical lymph nodes), distant metastasis, or development of a second primary cancer. There is certainly a need for a reliable blood test to determine prognosis in SCCHN patients, specifically those patients who may be at increased risk of locoregional recurrence or distant metastasis.

Cancer metastasis may develop when cells from the primary tumor become invasive, making their way into the surrounding lymphatic or vascular channels. Lymph node involvement may only be microscopic, and therefore not always clinically or radiographically evident. In cancer of the oral cavity, about 25% of patients will have microscopic disease in the lymph nodes, despite a clinically negative neck (4, 5). The

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presence of regional lymphatic metastasis in SCCHN is currently the most significant prognostic factor impacting survival, reducing survival by 50% (6–8). When cancer cells invade into the intravascular space, they are referred to as circulating tumor cells (CTCs). The exact mechanisms by which tumor cells leave the primary tumor, invade local tissues, and go on to develop in distant sites is not yet known.

From a clinical standpoint, the presence of CTCs may impact prognosis and treatment by providing: 1) early, definitive evidence of occult cancer spread, 2) a quantifiable risk factor for the development of distant metastatic disease, 3) the opportunity for further genetic analysis for targeted treatment strategies such as radiation or chemotherapy sensitivity, 4) a biomarker for post-treatment cancer surveillance. Studies have linked disseminated tumor cells to poor prognosis in breast (9), lung (10, 11), prostate (12), and colorectal cancers (13) but limited work has been done with regard to the role of CTCs in SCCHN.

While a milliliter of human blood contains an average of 5 billion RBCs, 7 million WBCs, and 295 million platelets, it is certainly a diagnostic challenge to identify a CTC (14). Researchers have taken several different approaches to accomplish this task. One approach is RT-PCR analysis of cancer-specific antigens, which are presumed to not be found in the blood, without visual cellular confirmation; this has been reported in SCCHN (15–19). Although this appears promising in melanoma (20–23), this technique may result in the detection of false positives due to non-specific amplification of genetic material. Other methods have used filtration to detect CTCs by size alone (24, 25), which implies that all CTCs are indeed larger than WBCs.

More commonly, the use of positive selection of CTCs by binding an antibody to a cancer-specific antigen on the cell surface has been performed. The most well-known are the CellSearch system by Veridex (26, 27) and the CTC-chip technology (28). Both of these methods rely on epithelial cell adhesion molecule (EpCAM) expression on the cell surface of CTCs in order to isolate and identify the cell (although other markers are also used for further analysis). While this is effective for identification of EpCAM positive cells, it may not be adequate for those cells which do not express this marker. The use of positive selection techniques for CTCs creates a significant intrinsic bias into the detection process since it is assumed that a CTC must express the cell surface marker targeted. Although such positive selection methods have not yet been described in the study of SCCHN, this potential diagnostic limitation has already been experimentally confirmed with another epithelial cancer, breast cancer. It has been demonstrated that the recovery of spiked breast cancer cells into the blood is a function of EpCAM expression. Those cell lines that highly expressed EpCAM were well-recovered using CellSearch; however other more aggressive breast cancer cell lines, which were high in CD44 expression, were poorly recovered, and 98% of these cells were missed (29). There is a growing body of evidence that epithelial to mesenchymal transition (EMT) takes place in the primary tumor, and it is possible that these cells may alter expression of their typical surface markers. EpCAM may be down-regulated during EMT, a process in which cancer cells acquire a more invasive and migratory phenotype (30–34).

In contrast to the positive selection approaches, we have developed an enrichment methodology which is based only on depletion of normal cells. We have demonstrated that this optimized methodology is able to obtain an average 5.66 $\log_{10}$, and as high as >7 $\log_{10}$ total enrichment of CTCs in the blood of patients with SCCHN (35). To date, we have not found any evidence of CTCs in healthy patients. Such a method leaves CTCs in their native state (without binding of surface antibodies) to allow further unaltered cellular characterization. The RBCs are removed by lysis, followed by magnetic separation of immunomagnetically labeled CD45+ leukocytes, leaving potential CTCs for further characterization. Immunocytochemical staining can then be performed using nuclear staining, cytokeratin, and other markers. Using confocal imaging, we have found a number of patients expressing cytokeratin positive CTCs (36), but also what appear to be tumor microemboli. Most interestingly, we have found nucleated cells with weak or no cytokeratin expression, but rather mesenchymal characteristics. It is tempting to speculate that such cells have undergone EMT and are in circulation in patients with SCCHN; certainly more research is needed to further define such CTC phenotypes. We have not found these cells in healthy human blood samples, and the biologic behavior of these cells in SCCHN is not known.

There is promising evidence that CTCs are predictive of prognosis in SCCHN. Although there is no visual confirmation of the presence of a CTC, RT-PCR studies such as mRNA expression of cytokeratin 20 in the blood of patients with oral cavity squamous cell carcinoma, have been linked to reduced disease-free survival and increased frequency of lymph node metastasis (16). Partridge et al. (2003) used a negative depletion methodology to identify disseminated tumor cells in SCCHN patients and correlated outcomes. They found that the detection of disseminated tumor cells pre-operatively or intra-operatively indicated an increased risk of local/distant recurrence and reduced survival (37). We have published our early prospective clinical results of 48 patients with SCCHN with a mean follow-up of 19 months, showing a statistically significant worse disease-free survival in patients with CTCs present at the time of surgical resection. We also observed that the presence of an increasing number of CTCs correlated with a worse outcome. There was no correlation between the presence of CTCs and lymph node metastasis, which suggests that with further study of additional patients, CTCs may turn out to be an independent prognostic marker in SCCHN (38).

We have developed a high-performance negative depletion technique for the identification of CTCs in the blood of patients with SCCHN, and our early clinical follow-up to date has been encouraging, suggesting that the presence of CTCs may be designated as a potential novel prognostic marker. The biologic mechanisms underlying CTCs and cancer recurrence or distant metastasis are not yet well-established, and until greater knowledge is acquired, techniques using cancer specific markers to potentially select out only a subpopulation of CTCs, may miss abnormal cells in cancer patients. With regard to CTCs, there are numerous unanswered questions, and we suggest that in order to objectively find answers, an unbiased approach may be best. With further clinical follow-up, we will continue to elucidate the long-term significance of CTCs in patients with SCCHN; certainly, larger prospective studies are warranted.

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References

1. Franceschi D, Gupta R, Spiro RH, Shah JP. Improved survival in the treatment of squamous carcinoma of the oral tongue. Am J Surg. 1993 Oct; 166(4):360–5. [PubMed: 8214293]
2. Zarbo RJ, Crissman JD. The surgical pathology of head and neck cancer. Semin Oncol. 1988 Feb; 15(1):10–9. [PubMed: 3278387]
3. Gath HJ, Brakenhoff RH. Minimal residual disease in head and neck cancer. Cancer Metastasis Rev. 1999; 18(1):109–26. [PubMed: 10505550]
4. McGuirt WF Jr, Johnson JT, Myers EN, Rothfield R, Wagner R. Floor of mouth carcinoma. The management of the clinically negative neck. Arch Otolaryngol Head Neck Surg. 1995 Mar; 121(3): 278–82. [PubMed: 7873143]
5. Yuen AP, Wei WI, Wong YM, Tang KC. Elective neck dissection versus observation in the treatment of early oral tongue carcinoma. Head Neck. 1997 Oct; 19(7):583–8. [PubMed: 9323146]
6. Jones AS, Roland NJ, Field JK, Phillips DE. The level of cervical lymph node metastases: their prognostic relevance and relationship with head and neck squamous carcinoma primary sites. Clin Otolaryngol Allied Sci. 1994 Feb; 19(1):63–9. [PubMed: 8174305]
7. Leemans CR, Tiwari R, Nauta JJ, van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck carcinoma. Cancer. 1993 Jan 15; 71(2):452–6. [PubMed: 8422638]
8. Snow GB, Annyas AA, van Slooten EA, Bartelink H, Hart AA. Prognostic factors of neck node metastasis. Clin Otolaryngol Allied Sci. 1982 Jun; 7(3):185–92. [PubMed: 7105450]
9. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med. 2005 Aug 25; 353(8):793–802. [PubMed: 16120859]
10. Pantel K, Izbicki J, Passlick B, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. Lancet. 1996 Mar 9; 347(9002):649–53. [PubMed: 8596379]
11. Kasimir-Bauer S, Schleucher N, Weber R, Neumann R, Seeber S. Evaluation of different markers in non-small cell lung cancer: prognostic value of clinical staging, tumour cell detection and tumour marker analysis for tumour progression and overall survival. Oncol Rep. 2003 Mar-Apr; 10(2):475–82. [PubMed: 12579292]
12. Kollermann J, Weikert S, Schostak M, et al. Prognostic significance of disseminated tumor cells in the bone marrow of prostate cancer patients treated with neoadjuvant hormone treatment. J Clin Oncol. 2008 Oct 20; 26(30):4928–33. [PubMed: 18794550]
13. Leinung S, Wurl P, Schonfelder A, Weiss CL, Roder I, Schonfelder M. Detection of cytokeratin-positive cells in bone marrow in breast cancer and colorectal carcinoma in comparison with other factors of prognosis. J Hematother Stem Cell Res. 2000 Dec; 9(6):905–11. [PubMed: 11177604]
14. McKenzie Textbook of Hematology. Baltimore, MD: Williams & Wilkens Inc.; 1996.
15. Wirtschafter A, Benninger MS, Moss TJ, Umiel T, Blazoff K, Worsham MJ. Micrometastatic tumor detection in patients with head and neck cancer: a preliminary report. Arch Otolaryngol Head Neck Surg. 2002 Jan; 128(1):40–3. [PubMed: 11784252]
16. Toyoshima T, Vairaktaris E, Nkenke E, Schlegel KA, Neukam FW, Ries J. Hematogenous cytokeratin 20 mRNA detection has prognostic impact in oral squamous cell carcinoma: preliminary results. Anticancer Res. 2009 Jan; 29(1):291–7. [PubMed: 19331164]
17. Ramani PT G, Ahmed S. Use of Rt-PCR in detecting disseminated cancer cells after incisional biopsy among oral squamous cell carcinoma patients. Journal of Cancer Research and Therapeutics. 2005; 1(2):92–7. [PubMed: 17998634]
18. Dyavanagoudar S, Kale A, Bhat K, Hallikerimath S. Reverse transcriptase polymerase chain reaction study to evaluate dissemination of cancer cells into circulation after incision biopsy in oral squamous cell carcinoma. Indian J Dent Res. 2008 Oct-Dec;19(4):315–9. [PubMed: 19075434]

19. Stathopoulou A, Ntoulia M, Perraki M, et al. A highly specific real-time RT-PCR method for the quantitative determination of CK-19 mRNA positive cells in peripheral blood of patients with operable breast cancer. Int J Cancer. 2006 Oct 1; 119(7):1654–9. [PubMed: 16708381]
20. Pinzani P, Mazzini C, Salvianti F, et al. Tyrosinase mRNA levels in the blood of uveal melanoma patients: correlation with the number of circulating tumor cells and tumor progression. Melanoma Res. 2010 Aug; 20(4):303–10. [PubMed: 20442676]
21. Arenberger P, Arenbergerova M, Vohradnikova O, Kremen J. Early detection of melanoma progression by quantitative real-time RT-PCR analysis for multiple melanoma markers. Keio J Med. 2008 Mar; 57(1):57–64. [PubMed: 18382126]
22. Gkalpakiotis S, Arenberger P, Kremen J, Arenbergerova M. Quantitative detection of melanoma-associated antigens by multimarker real-time RT-PCR for molecular staging: results of a 5 years study. Exp Dermatol. 2010 Nov; 19(11):994–9. [PubMed: 20812969]
23. Koyanagi K, O'Day SJ, Boasberg P, et al. Serial monitoring of circulating tumor cells predicts outcome of induction biochemotherapy plus maintenance biotherapy for metastatic melanoma. Clin Cancer Res. 2010 Apr 15; 16(8):2402–8. [PubMed: 20371696]
24. Vona G, Sabile A, Louha M, et al. Isolation by size of epithelial tumor cells : a new method for the immunomorphological and molecular characterization of circulatingtumor cells. Am J Pathol. 2000 Jan; 156(1):57–63. [PubMed: 10623654]
25. De Giorgi V, P P, Salvianti F, Panelos J, Paglierani M, Janowska A, Grazzini M, Wechsler J, Orlando C, Santucci, Lotti T, Pazzagli M, Massi D. Application of a filtration- and isolation-by-size technique for the detection of circulating tumor cells in cutaneous melanoma. Journal of Investigational Dermatology. 2010; 130(10):2440–7.
26. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004 Aug 19; 351(8):781–91. [PubMed: 15317891]
27. Riethdorf S, Fritsche H, Muller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res. 2007 Feb 1; 13(3):920–8. [PubMed: 17289886]
28. Stott SL, Hsu CH, Tsukrov DI, et al. Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. Proc Natl Acad Sci U S A. 2010 Oct 26; 107(43):18392–7. [PubMed: 20930119]
29. Sieuwerts AM, Kraan J, Bolt J, et al. Anti-epithelial cell adhesion molecule antibodies and the detection of circulating normal-like breast tumor cells. J Natl Cancer Inst. 2009 Jan 7; 101(1):61–6. [PubMed: 19116383]
30. Berx G, Raspe E, Christofori G, Thiery JP, Sleeman JP. Pre-EMTing metastasis? Recapitulation of morphogenetic processes in cancer. Clin Exp Metastasis. 2007; 24(8):587–97. [PubMed: 17978854]
31. Chaffer CL, Thompson EW, Williams ED. Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs. 2007; 185(1–3):7–19. [PubMed: 17587803]
32. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008 May 16; 133(4):704–15. [PubMed: 18485877]
33. Sarrio D, Rodriguez-Pinilla SM, Hardisson D, Cano A, Moreno-Bueno G, Palacios J. Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. Cancer Res. 2008 Feb 15; 68(4):989–97. [PubMed: 18281472]
34. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004 Jun 25; 117(7):927–39. [PubMed: 15210113]
35. Yang L, Lang JC, Balasubramanian P, et al. Optimization of an enrichment process for circulating tumor cells from the blood of head and neck cancer patients through depletion of normal cells. Biotechnol Bioeng. 2009 Feb 1; 102(2):521–34. [PubMed: 18726961]
36. Balasubramanian P, Yang L, Lang JC, et al. Confocal images of circulating tumor cells obtained using a methodology and technology that removes normal cells. Mol Pharm. 2009 Sep-Oct;6(5): 1402–8. [PubMed: 19445481]
37. Partridge M, Brakenhoff R, Phillips E, et al. Detection of rare disseminated tumor cells identifies head and neck cancer patients at risk of treatment failure. Clin Cancer Res. 2003 Nov 1; 9(14): 5287–94. [PubMed: 14614011]

38. Jatana KR, Balasubramanian P, Lang JC, et al. Significance of circulating tumor cells in patients with squamous cell carcinoma of the head and neck: initial results. Arch Otolaryngol Head Neck Surg. 2010 Dec; 136(12):1274–9. [PubMed: 21173379]