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Tracking the Origin of Metastatic Prostate Cancer

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Abstract

Metastatic prostate cancer is a monoclonal disease. We previously failed to identify a common somatic denominator between primary tumor tissue and two lymph-node metastases by exome sequencing [Lindberg J, et al. Eur Urol 2013;63:702–8]. To track the seeding clone we performed copy-number alteration analysis on 34 morphologically distinct tissue areas in one prostatectomy specimen. Using break-point regions to infer phylogenetic relationships, the clone most closely related to the metastases was found in intraductal carcinoma of the prostate. Although the majority of tumor areas harbored events also found in the metastases, three carried none. This emphasizes the importance of intraprostatic tumor heterogeneity for prediction of prognosis. These findings also support recent evidence that intraductal carcinoma is a marker of aggressive disease.
Patient summary: We identified the area in the prostate that gave rise to metastases by searching for metastatic-specific DNA alterations in multiple regions of the prostate. The metastasizing component grew within prostatic ducts, suggesting that intraductal cancer should be reported when found in needle biopsies. It is also important to be aware of tumor heterogeneity when assessing somatic changes linked to tumor aggressiveness.

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Prostate-specific antigen screening saves lives by lowering the probability of dying from prostate cancer [1]. Nevertheless, many indolent age-related cancers are treated without any apparent benefit for the patient. Therefore, the currently direst aim of clinical prostate cancer research is to establish an improved treatment rationale at primary diagnosis. Histopathological appearance and Gleason grading are currently important tools in risk stratification and management of localized prostate cancer. However, personalized genomic profiling for selection of the most efficient treatments is the future of clinical practice. Although conceptually demonstrated by RNA profiling for assessment of proliferation rates [2], clinical implementation is limited by pronounced intraprostatic tumor heterogeneity. The majority of prostate cancers are multifocal [3], and we previously used whole-exome sequencing to demonstrate that spatially separated tumor foci are somatically independent [4]. This is in contrast to metastatic prostate cancer, which is of monoclonal origin [5], and has implications for any approach using tumor biopsies for prognostication.

As part of a genomics study investigating correlations between different tumor characteristics that was published

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in this journal [6], we set out to verify the monoclonal relationship between primary tumor tissue and metastases by exome sequencing in two patients. The first patient carried an aggressive neuroendocrine tumor that exhibited a clear monoclonal relationship to bone metastasis. Surprisingly, in the second patient (SWE-54), we could not find any common somatic denominator between fresh-frozen primary tumor tissue obtained after radical prostatectomy (SWE-54A) and fresh-frozen right/left pelvic lymph-node metastases (SWE-54-B/C). Therefore, in an attempt to identify the clone seeding the metastases, we conducted laser-capture microdissection of 45 morphologically distinct areas using formalin-fixed paraffin embedded tissue blocks (Supplementary Text, Supplementary Fig. 1, Supplementary Table 1). For tracking of the metastases we adopted a similar strategy as Navin et al. [7], performing low-pass whole-genome sequencing to detect break-point regions (BPRs), marking the start of an amplification or a deletion event (Supplementary Text). The presence of BPRs was used to infer phylogenetic somatic relationships. Eleven areas were lost during tissue harvesting, library preparations, or quality control of the data. Additionally, nine tissue areas contained less than five BPRs and were excluded, leaving 25 tumor areas for further analysis. In total, 385 BPRs were identified as being present in two or more tumor areas. These BPRs were used to construct a phylogenetic tree (Fig. 1A) and the somatic relationships were visualized by coloring the proportion of BPRs shared with metastases (Fig. 1B, Supplementary Text). Remarkably, tissues with the highest level of similarity to the lymph-node metastases were the three areas where an intraductal carcinoma (IDC) component was microdissected for sequencing (Supplementary Figs. 2 and 3). To verify the diagnosis of IDC, immunohistochemistry for p63 and alpha-methylacyl-CoA-racemase (AMACR, p504s) was performed. All glandular elements of the IDC areas had either a complete or fragmented basal cell layer, confirming the morphological diagnosis of IDC (Fig. 2A,B). Interestingly, one area of

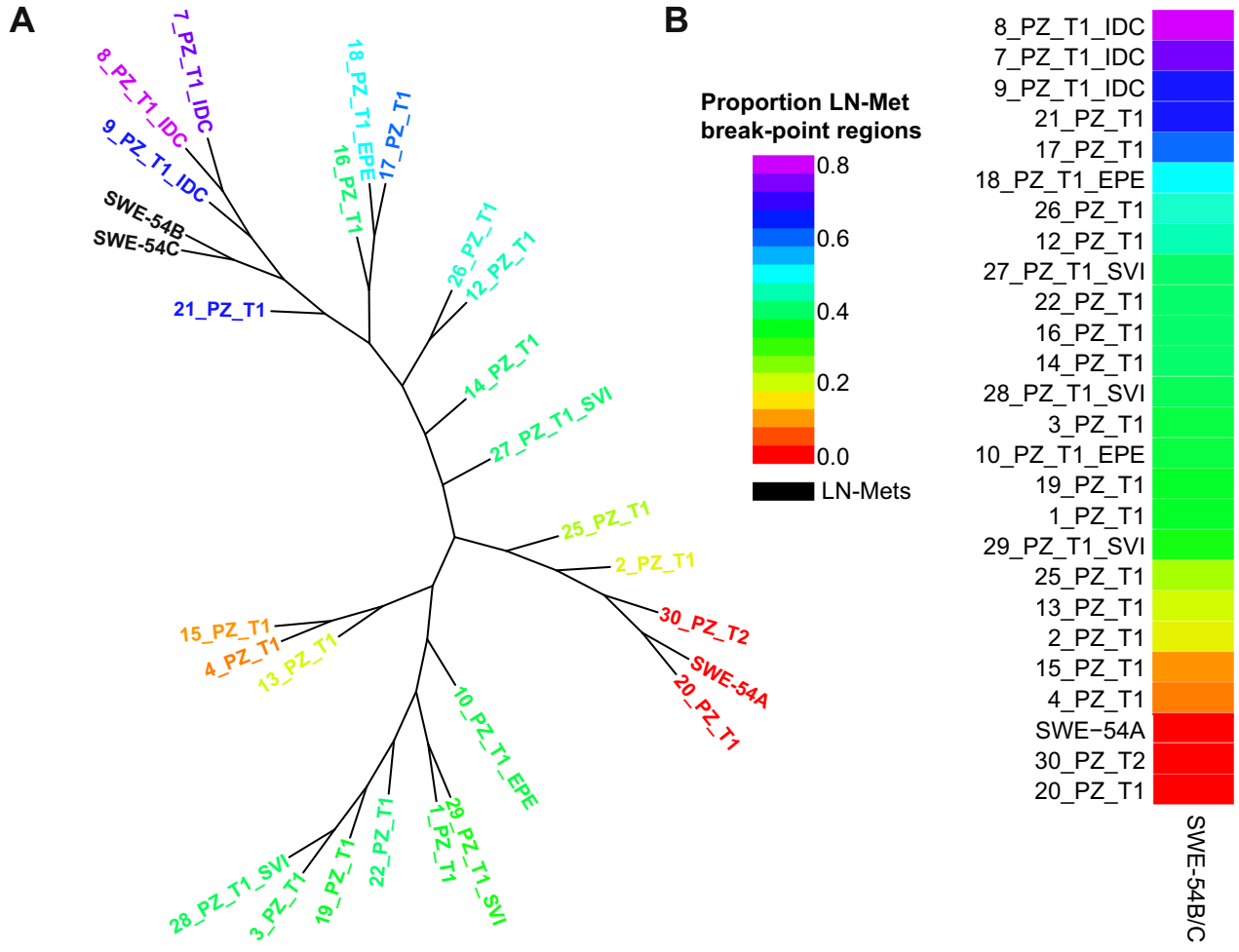

Fig. 1 – The somatic relationships of the tumor areas. (A) A phylogenetic tree was constructed using a neighbor-joining algorithm to track the clone from the primary tumor tissue that seeded the metastases. (B) To visualize the somatic relationship to metastases, samples were colored according to the proportion of break-point regions shared with lymph-node metastases. SWE-54A (primary fresh-frozen tissue), SWE-54B (right fresh-frozen lymph-node metastasis), and SWE-54C (left fresh-frozen lymph-node metastasis) represent tissues previously profiled by low-pass whole-genome sequencing and whole-exome sequencing [1]. Tumor areas are labeled according to area, zone, focus, and additional annotation; for example, 8_PZ_T1_IDC corresponds to area 8, peripheral zone, tumor focus 1, intraductal carcinoma. TZ = transition zone; PZ = peripheral zone; IDC = intraductal carcinoma; EPE = extraprostatic extension; SVI = seminal vesicle invasion.