Cancer Genetics 207 (2014) 206–213

Cancer Genetics

Predictive chromosomal clusters of synchronous and metachronous brain metastases in clear cell renal cell carcinoma

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Synchronous (early) and metachronous (late) brain metastasis (BM) events of sporadic clear cell renal cell carcinoma (ccRCC) (n = 148) were retrospectively analyzed using comparative genomic hybridization (CGH). Using oncogenetic tree models and cluster analyses, chromosomal imbalances related to recurrence-free survival until BM (RFS-BM) were analyzed. Losses at 9p and 9q appeared to be hallmarks of metachronous BM events, whereas an absence of detectable chromosomal changes at 3p was often associated with synchronous BM events. Correspondingly, k-means clustering showed that cluster 1 cases generally exhibited low copy number chromosomal changes that did not involve 3p. Cluster 2 cases had a high occurrence of −9p/−9q (94–98%) deletions, whereas cluster 3 cases had a higher frequency of copy number changes, including loss at chromosome 14 (80%). The higher number of synchronous cases

Received January 23, 2014; received in revised form May 1, 2014; accepted May 10, 2014.

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http://dx.doi.org/10.1016/j.cancergen.2014.05.004

in cluster 1 was also associated with a significantly shorter RFS-BM compared with clusters 2 and 3 ($P = 0.02$). Conversely, a significantly longer RFS-BM was observed for cluster 2 versus clusters 1 and 3 ($P = 0.02$). Taken together, these data suggest that metachronous BM events of ccRCC are characterized by loss of chromosome 9, whereas synchronous BM events may form independently of detectable genetic changes at chromosomes 9 and 3p.

Keywords Clear cell renal cell carcinoma, brain metastasis, comparative genomic hybridization (CGH), chromosomal copy number alterations, tumor progression

Renal cell carcinoma (RCC) constitutes only 2% of all cancers (1), yet accounts for 7–10% of brain metastasis (BM) cases (2,3). Moreover, following treatment of localized RCC, approximately 20–30% of patients reportedly develop distant metastasis (4). Depending on the extent of the primary disease, BM has developed in 2% of patients who exhibited exclusive abdominal spread, and in up to 16% of patients with concurrent pulmonary and bone metastasis of RCC (5). Once BM is evident, the effectiveness of any palliative systemic therapy is very restricted due to the increased risk of side effects, such as cerebral hemorrhage (5–12). In fact, BM with RCC is an independent negative prognostic factor for survival (13). It has been reported that if the BM is untreated, the mean survival time for patients with BM from RCC is 3 months. The survival period increases to 15.5 months if brain surgery and whole brain radiotherapy are applied (14); however, the onset of BM is unpredictable, with the interval between a primary diagnosis of RCC and BM ranging from a concomitant diagnosis to >20 years (15–18).

Metastasis to distant sites in RCC is believed to be a highly selective, non-random process that comprises a series of sequential genetic events (19,20). Histologically and genetically, RCC is a heterogeneous disease that can be subdivided into clear cell (ccRCC), papillary, chromophobe, and mixed cell variants (19,20). ccRCC is the most frequent type (70–78%) (21,22). Data from genome-wide genetic analyses, including classic cytogenetics, comparative genomic hybridization (CGH), and cytogenomic arrays, suggest that losses at 3p and 14q, as well as gains at 5q, may be critical aberrations in the development of ccRCC (23–27). Furthermore, chromosomal losses at 4, 9, 13q, 14q, and 18, and chromosomal gains at 1q, 7, and 17, have been associated with more aggressive variants of ccRCC (25,27–32). Unfortunately, data regarding chromosomal changes in BM events of RCC are rare, and are currently restricted to case reports. To date, four cases (33) and another single case (29) have been reported. In contrast, no information regarding histomorphological subtypes of RCC have been published.

The aim of this study was to analyze chromosomal imbalances in 148 cases of BM of ccRCC as identified by CGH with respect to clinical follow-up data of recurrence and survival. Probabilistic oncogenetic models were subsequently applied to these data. These models allowed multiple cytogenetic pathways to occur simultaneously within a given data set, without assuming a unique order of events (34).

Materials and methods

Patients and clinical data

Ethics committee approval was obtained for all cases included in this study. The data set included 148 cases of ccRCC BM that were treated at 11 different neuropathology institutions of the Universities of Berlin, Brandenburg, Bremen, Erlangen, Essen, Göttingen, Greifswald, Homburg, Leipzig, Münster, and Bremen in Germany. For these cases, CGH was retrospectively performed using formalin-fixed, paraffin-embedded BM tissue samples. In addition, all cases exhibiting ccRCC histomorphology were re-evaluated by an expert pathologist (L.F.). Recurrence-free survival following diagnosis and surgery for ccRCC with BM (RFS-BM) was also evaluated. Cases were categorized as synchronous (RFS-BM $\leq$ 1 months) or metachronous (RFS-BM $>$ 6 months) BM, and were defined according to the presence or absence of BM at the time of the initial diagnosis of the primary ccRCC. No BM events occurred within 2–6 months of diagnosis. Overall survival (OS) was calculated from the initial diagnosis of the primary tumor to death.

CGH

DNA preparation and CGH

Tumor DNA was extracted from formalin-fixed, paraffin-embedded BM samples using proteinase K digestion (2 mg/mL final concentration; Roche, Mannheim, Germany) followed by spin column purification (Qiagen, Hilden, Germany). As a reference, pooled normal DNA from the opposite gender was used as an internal quality control. Labeling of tumor DNA was performed with biotin-16-dUTP nick translation (Roche, Mannheim, Germany) and digoxigenin-11-dUTP (Roche, Mannheim, Germany) as a normal DNA reference. Denatured DNA probes containing 2 μg tumor DNA, 1.5 μg reference DNA, and 80 μg COT-1 DNA were hybridized to normal metaphase spreads on glass slides (15 × 15-mm cover glass area) for 3 days. The slides were subsequently washed, blocked with bovine serum albumin solution, and incubated with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA) and rhodamine-conjugated antidigoxigenin (Roche). Finally, the slides were washed and mounted in antifade solution (Vector Laboratories), which contained 2.5 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) counterstain.

Imaging and image analysis

Image acquisition was performed using a Zeiss Axioskop fluorescence microscope (Zeiss, Göttingen, Germany) equipped with three separate bandpass filters (DAPI bandpass, green single bandpass, and a red single bandpass) and a high-sensitivity monochrome charge coupled device (CCD) camera (Photometrics, Tucson, AZ). For each analysis, the mean chromosome-specific green-to-red fluorescence ratios and associated 95% confidence intervals (CIs) from at least 10 well-selected metaphases were plotted using Quips CGH software (distributed by Applied Imaging,