ABSTRACT
Local and distant tumor spread was evaluated and compared with DNA content analyzed by flow cytometry of eight samples from each of 71 renal cell carcinomas. Twenty-six tumors were homogeneously diploid while 45 tumors contained at least one aneuploid tumor sample. Diploid tumors generally respected the surrounding tissues and only three of 26 tumors (12%) had evidence of local tumor invasion. In contrast, 33 of 45 patients (73%) with aneuploid tumors had local invasion (p < 0.001).

INTRODUCTION
The ability of malignant cells to invade normal tissue and penetrate into lymphatic and blood vessels as well as to spread to near and distant sites is a characteristic feature of a neoplastic process. Various components of the metastatic character have been described, although interactive processes and mechanisms occur during tumor progression. As defined by Foulds (1), tumor progression is a neoplastic development manifested by permanent, irreversible changes in one or several tumor characteristics. Tumor DNA content is one variable with measurable quantitative changes which has given valuable prognostic information in renal cell carcinoma (2–4). Patients with diploid tumors generally have better prognosis than those with aneuploid tumors. We have previously noticed a higher frequency of aneuploidy in more advanced local stages for patients without distant metastases at diagnosis (5). However, for patients with distant metastases the frequency of tumors with diploid and aneuploid DNA content was about equal (3).

Data on renal cell carcinoma with respect to tumor spread in relation to DNA content has not previously been presented. We report here on tumor invasion, metastatic spread, and DNA content in a series of 71 consecutive patients with renal cell carcinoma.

MATERIALS AND METHODS
Patients. Seventy-five patients were nephrectomized for renal cell carcinoma from January 1982 to February 1987, at the Urologic Clinic, University Hospital, Umeå. Four of these patients were excluded from the study; two patients due to total necrosis of the primary tumor and two due to technical reasons. Thus, 71 patients, 43 men and 28 women were included in the study. Their mean age was 63.4 years, range 28 to 81 years. Extensive perifascial nephrectomy was performed in 69 patients and partial nephrectomy in two. Lumbar lymph node dissection of varying extent was performed in 39 patients.

Tumors. The nephrectomized kidney with tumor was divided. One kidney cortex sample and eight tumor samples were taken in each case as described previously (6). Each sample was divided into two parts, one for histopathological examination and one for flow cytometric DNA analysis.

Histopathological Examination. Histopathological grading was performed using a scale from 1 to 4 (7). The examination included, apart from the eight tumor samples primarily taken, microscopic evaluation of local tumor invasion using the parameter's sharp or diffuse demarcation of the tumor to adjacent kidney cortex tissue, through the renal capsule into the perirenal fat and presence of tumor invasion into renal veins in the hilar region. Histopathologically diffuse or infiltrating demarcation of the tumor to adjacent tissues was judged as one feature of local invasion. The ipsilateral adrenal, dissected lymph nodes and available metastases were also histopathologically examined. Invasion of tumor cells into blood vessels in the tumor borderline zone was analyzed in 66 tumors.

Clinical Staging. Clinical Stage (I–III) was defined according to extent of spread: Stage I, tumors confined within the renal capsule; Stage II, spread outside the renal capsule into perinephric fat or tumor involving the renal vein, or spread to the ipsilateral adrenal or regional lymph nodes; Stage III, evidence of distant metastases or extension to adjacent organs. Distant metastases were evaluated in all patients by clinical and radiological investigations, and by inspection and palpation of the intra- and retroperitoneal tissues and organs during the operation. The preoperative radiological examination consisted of pulmonary X-ray, angiography, computerized tomography, ultrasonography, and bone scans.

DNA Analysis. The method for flow cytometric DNA analysis has been described in detail previously (6). Briefly, the fresh samples were minced and stained using a propidium iodide solution containing a detergent (8). After staining, the samples were filtered and run in a model 4800A cytofluorograph (Biophysics System Inc., NY) or for the last 27 tumors in an FACS Analyzer (Becton-Dickinson, Sunnyvale, CA). The kidney cortex tissue samples were used as standards for diploidy. The tumor samples were denominated diploid (DNA index, 1.0) when only one peak was detected and aneuploid when two separate peaks were found, since it was assumed that all tumor samples contained normal as well as tumor cells. Cell populations at or near 4c were judged as true tetraploid if a definitive S-phase could be visualized in the 8c region. The 4800A cytometer had a coefficient of variation of 4–4.5% and the FACS Analyzer a coefficient of variation of ≤3% for normal lymphocytes. A primary tumor was denominated diploid when all eight tumor samples were diploid and accordingly a tumor was aneuploid when at least one of the eight samples was aneuploid.

The χ2 test was used for statistical analysis.

RESULTS
DNA Content and Histopathological Grade. The distribution of DNA content in relation to histopathological grade is presented in Table 1. The frequency of patients with aneuploid tumors was 63% with DNA indices varying from 1.3 to 2.8. A relationship between DNA content and histopathological grade was found (Table 1). However, Grade 3 tumors were most common and had about the same proportion in diploid and aneuploid tumors (46 and 62%, respectively).

DNA Content and Clinical Stage. DNA content in relation to
clinical stage is summarized in Table 2. Patients with diploid tumors had mostly Stage I tumors (57%). Among 21 tumors in Stage II, 19 (90%) had an aneuploid primary tumor while two tumors were diploid. About one third of patients both with diploid and those with aneuploid primary tumors had distant metastases (Stage III).

DNA Content and Local Tumor Invasion. Local tumor invasion and DNA content are presented in Table 3. The studied parameters of local tumor invasion showed a significant difference between diploid and aneuploid tumors (p < 0.001). In total, three of 26 diploid tumors (12%) showed at least one parameter of local tumor invasion. On the contrary, 33 of 45 aneuploid tumors (73%) had local tumor invasion (Table 3). Invasion of tumor cells into blood vessels in tumor tissue at the tumor borderline zone was found in four of 25 diploid and in 21 of 41 aneuploid tumors (16 and 51%, respectively).

DNA Content and Local Metastases. The metastatic spread to the ipsilateral adrenal and regional lymph nodes is shown in Table 4. In 12 patients with diploid primary tumors lymph node dissection and in 23 examination of the ipsilateral adrenal was performed, without found of any metastases. In contrast, metastatic spread to lymph nodes was found in seven of 25 patients (28%) with aneuploid primary tumors. Six of these seven patients (86%) also had distant metastases. Of the remaining 18 patients with aneuploid tumors and without lymph node metastases, only one (6%) had distant metastases. In three of 39 patients with aneuploid primary tumor, metastatic spread to the ipsilateral adrenal was found. These three patients also had distant metastases. In total, local metastases were found in nine patients, all having aneuploid primary tumors as well as local tumor invasion.

DNA Content and Distant Metastases. Nine of 26 patients (35%) with diploid tumors had distant metastases (Tables 2 and 5). Eight of these nine patients had no sign of local tumor invasion. In patients with aneuploid tumors, 13 of 45 (29%) had distant metastases. In contrast to the diploid tumors, all 13 aneuploid tumors with distant metastases had local tumor invasion (Tables 2 and 6). Lymph node dissection had been performed in nine of these 13 patients. Eight patients (89%) had local metastases while one had no lymph node metastases.

Six of nine patients with diploid primary tumors and distant metastases had solitary metastases, compared with two of 13 patients with aneuploid primary tumors (p < 0.02). Five of the nine patients with diploid tumors had skeletal and two had lung metastases. In the vagina, contralateral adrenal, liver and brain addition metastases were found. In patients with aneuploid tumors, six of 13 had skeletal metastases while 10 had lung metastases. Additional metastases in these patients were located in lymph nodes, contralateral adrenal, and liver. The distribution of lung metastases differed significantly (p < 0.02) between patients with diploid and aneuploid primary tumors.

**DISCUSSION**

The acquisition of the capacity to invade adjacent tissues and to metastasize is the most important clinical aspect of tumor progression. Attempts have been made to define specific properties of metastatic cells including production and secretion of proteolytic enzymes, plasminogen activators, tumor angiogenesis factors and growth factors (9–11). Biological and clinical tumor progression might reflect the appearance within a tumor of an increasing number of altered subpopulations with new characteristics (12). Concerning tumor cell DNA content, we have previously demonstrated a frequent heterogeneity in renal cell carcinoma (5) and due to this heterogeneity, we routinely analyze eight different samples from each tumor. Rabes et al. (13) have shown, by autoradiography, a striking proliferative heterogeneity within renal cell carcinoma with subpopulations of high proliferative activity at the invasive zone. Patients with diploid tumors survive significantly longer than patients with aneuploid tumors who generally have a rapid progression of the disease (2–4) and aneuploid tumors have significantly higher S-phase values (5, 14). Differences in proliferation rate and local spread between these tumor clones might be of importance for the course of the disease.

In the present study, diploid tumors generally respected the surrounding tissues. In contrast, aneuploid tumors were frequently associated with local tumor invasion, i.e., one of the characteristics of tumor progression. Diploid tumors had 12% local invasion compared with 73% in aneuploid tumors. The finding that aneuploid tumors more frequently invaded into renal veins is interesting, since it has been demonstrated that invasion of the renal vein by itself does not worsen the prognosis (7, 15). In addition, we found a significant difference in occurrence of local metastases. All patients with local metastases to regional lymph nodes or ipsilateral adrenal had aneuploid primary tumors as well as concomitant local invasion. Thus, local invasion and local metastases seem to be associated with aneuploidy. Our findings support the results from a prostate cancer study showing increased frequency of aneuploidy with advancing local stage (16).

Other factors than those of importance for local tumor invasion and spread might be involved in the complex phenomenon of tumor spread with distant metastases. Our results show that diploid tumors had metastasized, at the time of diagnosis, in about the same frequency as aneuploid tumors. Thus, in this
Although the ability to spread with distant metastases was equal for diploid and aneuploid tumors, sites and number of metastases differed. Diploid tumors more often had solitary metastases and most interestingly, lung metastases seemed to be a characteristic for aneuploid tumors.

Thus, valuable information of the behavior of renal cell carcinoma can be obtained by analysis of the DNA content. Diploid tumors respect the surrounding tissues as a rule while aneuploid tumors have a high frequency of local invasion and local metastases. The differences in tumor spread between diploid and aneuploid tumor clones might express the differences in clinical course. However, the DNA content of renal cell carcinoma cannot predict the occurrence of distant metastases, but distribution of metastases seems to differ between tumors with diploid and aneuploid DNA content.

REFERENCES


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aspect of tumor progression, diploid and aneuploid tumors seem to have equal malignant properties. However, our previous study shows that patients with distant metastases and diploid tumors survive significantly longer than those with aneuploid tumor, and DNA content of metastases seem to give additional prognostic information (3).

Invasion of tumor cells into the lymphatic and blood circulatory systems has been claimed to be the pathways for tumor spread with distant metastases. The vasculature of many solid tumors probably provides the direct entry for tumor cells into the venous system, and it has been proposed that this occurs mainly into the tumor vasculature at the level of the tumor capsule of a solid tumor (17). It seems possible that diploid tumors metastasize to distant sites via the blood stream, since, in our study, these tumors had low frequency of local invasion and local metastases. However, the diploid tumors also had a low frequency of invasion into blood vessels. Aneuploid tumors had a significantly higher frequency of invasion than the renal veins and tumor vessels and thus seem to have a direct entrance into the blood circulatory system. On the other hand, all patients with aneuploid primary tumors and distant metastases had concomitant local invasion and mostly also local metastases. Thus, aneuploid tumors might as well metastasize via local invasion and lymphatic drainage, and this might be one pathway for distant spread of these tumors. In fact, in patients without distant metastases and with aneuploid primary tumors, only one of 18 patients (6%) had metastatic spread to regional lymph nodes. Thus, it seems possible that the pathways for spread with distant metastases might differ between diploid and aneuploid tumors.
Tumor Spread and DNA Content in Human Renal Cell Carcinoma

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