Molecular background of urachal carcinoma

Ph.D. theses

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1. INTRODUCTION

The urachus is an embryonal remnant of the sinus urogenitale stretching between the anterior dome of the bladder and the umbilicus. It degenerates into a closed canal during embryonal development; however, this process may preform incomplete up to one third of cases. The remaining urachal remnant may give rise to urachal cancer (UrC).

UrC is a rare but highly malignant entity, usually detected due to its invasion to the urinary bladder. UrC accounts for 0.5% of bladder tumors and 30% of primary bladder adenocarcinomas (ADC). The most common histological type of UrC is ADC that shows several histological and molecular similarities with colorectal adenocarcinomas (CRC). The prognosis of UrC is poor; less, than 50% of UrC patients survive for 5 years. The main reason for this devastating prognosis is that UrC is mostly discovered at progressed stages due to its late symptoms.

The two most often used stage classifications are the so-called Sheldon- and Mayo- staging systems. There is no consensus regarding the applicability and prognostic value of these two staging systems.

The recommended treatment for local UrC is partial cystectomy with en bloc resection and removal of umibilical ligament and the umbilicus.

Due to late symptoms, 20-30% of UrC patients are diagnosed with metastatic stage. In lack of effective radiotherapy, systematic therapy is the only treatment option to prolong survival in these patients. Because of the rare occurrence of UrC, large-scale prospective clinical studies and treatment guidelines are not available. As a result chemotherapy regimens used both in urothelial cancer (cisplatin-based chemotherapy) and CRC (5-FU-based) are frequently used. The 5-year overall survival rate for patients with metastatic UrC is less than 20% highlighting the need for more effective systemic therapies. Our knowledge on UrC's clinicopathological feature and chemotherapeutic treatment is originating from case reports and single-institutional retrospective studies with low patient numbers. In order to summarize these published experiences, we performed a literature review and a meta-analysis of the published clinical and pathological data on UrC.

Deeper insight into the genetic background of UrC may help to improve therapy-decisions for UrC patients

Based on the histopathological and genetic similarity between CRC and UrC, targeted therapies effectively used in CRC may be

considered for the treatment of UrC. Anti-EGFR therapies are widely used as second-line treatments in CRC.

Mutation analysis of the Epidermal Growth Factor Receptor (EGFR) pathway members are widely used in the therapeutic decision-making. Some mutations along this signal transduction pathway result in continuously active signaling independent of upstream activation, leading to increased proliferation and survival of tumor cells. Specific mutations as well as the amplification of the EGFR gene are known as positive predictors for anti-EGFR therapy, while other activating hot-spot mutations along the EGFR pathway contraindicate EGFR-inhibitor therapies. In UrC, the few published results with EGFR-targeted therapy are promising; however, the prevalence and prognostic significance of the mutations of EGFR pathway are unknown.

Despite the great clinical relevance of the differential diagnosis between UrC and primary bladder ADC as well as CRC there are only few studies are available focusing on the immunohistochemical (IHC) characteristics of UrC. . Based on these publications CK20+, CK7+ or CK7-, cytoplasmic β-catenin staining and diffuse expression of CK-34βE12 may suggest the presence of UrC.

2. OBJECTIVES

2.1. In order to gain a more detailed insight into the clinicopathological features, prognosis and therapeutic options of UrC, we performed a comprehensive literature review and a meta-analysis of published clinical and pathological data of UrC. Our further purpose was to compare the effectivity of the different chemotherapeutic treatments.

2.2. A further aim was to collect a large number of UrC tissue samples and clinical data and to analyze them for the most frequently occurring mutations in the KRAS, BRAF, NRAS, EGFR and PIK3CA genes in CRC. We were looking for correlations between the mutation status and UrC patients' clinicopathological features and survival.

In addition we compared our results with published data on UrC's, CRC's, urothelilal cancer's and primary bladder ADC's mutation patterns.

2.3. For further information on the molecular background of UrC's we performed immunohistochemical staining for five proteins (Ki67, p53, MMP7, IMP3, RHAMM, BGN) with proven prognostic value in urothelial and colorectal carcinoma.

3. METHODS

3.1. Clinicopathology, prognosis and therapy of UrC

A systematic PubMed search on UrC has been performed using the terms “urachus”, “urachal cancer” and “urachal carcinoma” resulting in the identification of 25 studies with an overall number of 1010 UrC patients.

3.2. Mutation analysis of the EGFR signaling pathway

For mutation analysis, we collected 31 UrC formalin-fixed paraffin embedded (FFPE) samples from 5 university centres (Budapest, Essen, Cracow, Rennes and Vancouver). Tumor containing areas were marked on hematoxylin and eosin (HE) sections by a pathologist. DNA was isolated from dissected tumor tissues and were amplified by polymerase chain recation (PCR) for 13 exons of the KRAS, BRAF, NRAS, EGFR and PIK3CA genes. Mutations were identified by using the pyrosequencing method.

Generated results were combined results with those of published data to calculate mutation frequencies in UrC. These mutation frequencies were than compared to those of CRC, urothelilal and primer bladder ADC

3.3. Immunohistochemical examinations of UrC

IHC analysis for Ki67, p53, RHAMM, BGN, MMP-7 and IMP3 was performed on 15 UrC tissue samples.

Combined quantitative and qualitative evaluation was performed by 2 independent pathologist who were blinded to the clinical and follow-up data. For semiquantitative evaluation of RHAMM, BGN, MMP-7 and IMP3 staining the H-score has been applied. . Immunostaining of Ki67 and p53 was evaluated by assessing the rate of positive tumor nuclei.

4. RESULTS

4.1. Clinicopathology, prognosis and therapy of UrC

Proceed to systematic Pubmed search we performed comparative meta-analysis including 1010 UrC patients. Our results showed that UrC is more frequent in men (604/1010; 60%) than in women (406/1010; 40%). The median age was 52 years (range: 20-90 years). The most frequent symptoms were macroscopic or microscopic haematuria (73%), abdominal pain (14%), dysuria (13%) and mucosuria (10%). The most common histological type was ADC (90%). Twenty-one percent of patients had metastatic disease at diagnosis. Those cases that were treated surgically including lymph node resection, lymph node positivity was found in 17%. Surgical margin was positive in 21% of cases.

In multivariable analysis the following parameters were proved to be independent prognostic factors for survival: (1) Sheldon staging, (2) Mayo staging, (3) presence of positive lymph nodes, (4) presence of distant metastases, (5) positive surgical margin, (6) ECOG performance status.

To compare the efficiacy of the most often used chemotherapeutic agents we summerized available data on 74 UrC patients' chemotherapy treatment. Patients who were treated with 5-FU or with the combination of 5-FU and platinum showed the

highest response rates; 44% and 43% respectively. In contrast, the lowest response rate of 10% was found in patients who received platinum-based chemotherapy . In addition, the lowest progression rate was found in patients who were treated with platinum-5FU combinaton (14%). Taking together, the highest response rate with the lowest progression rate could be achieved when combining 5-FU with platinum.

4.2. Mutation analysis of the EGFR signaling pathway

We collected 31 urachal carcinoma samples from five universities and screened for the most commonly affected mutational hotspots in 13 exons of KRAS, NRAS, BRAF PIK3CA and EGFR genes. We found 14 mutations in 13 of 31 (42%) patients. The most frequently mutated genes were KRAS (26%) followed by BRAF (16%) and NRAS (3%). No mutations were found in EGFR and PIK3CA genes. We found no correlation between any of the mutations and patients' clinicopathological parameters (Sheldon staging, Mayo staging, grading, presence of lymph node metastases, calcification and signet ring cell phenotype). Furthermore, there was no association between any of the mutations and patients' survival.

Our comparative analysis of mutational patterns between UrC, primary bladder ADC and CRC revealed the frequent occurrence

of KRAS in both UrC and CRC (28% vs. 41%). A further similar mutational frequency between UrC and CRC was found regarding the BRAF gene (8% in UrC and 10% in CRC), while NRAS and EGFR genes were rarely affected in both cancers. The mutation frequencies of UrC showed a characteristic difference to that of urothelial bladder cancer; KRAS mutations were found to be frequent in UrC in contrast to urothelial cancer (3%). Furthermore, PIK3CA genes was affected often in urothelial cancer (20%) while PIK3CA mutations were absent in UrC. The comparison between UrC and primary bladder ADCs revealed several similarities; KRAS gene is often affected both in UrC (28%) and in primary bladder ADC (18%). Furthermore, similar mutational frequencies were detected in the BRAF gene (8% in both entities), while NRAS and EGFR mutations are rare or absent in both tumors.

4.3. Immunohistochemical examinations of UrC

IHC analysis revealed higher expressions of IMP3 and RHAMM in UrC compared to adjacent normal tissue (p=0,0431 and p=0,0052). Intensity of Ki67 and p53 staining were significantly higher in tumor tissue (p=0,0006 and p=0,0024 respectively). In addition, IMP3 were stronger expressed in low stage UrC (Sheldon ≤ IIIA; p=0,0048). None of the analysed markers were associated with tumors' grading and patients' gender

or age. MMP-7, BGN, RHAMM, IMP3, Ki67 and p53 immunoreactivity results showed no significant influence on survival in the Cox regression analysis.

5. CONCLUSIONS

5.1. Our literature review revealed that 5-FU-based chemotherapies (used in CRC) are superior to those of platinum-based regimens (used in urothelial cancer) for the chemotherapeutic treatment of UrC. The best therapy response can be achieved when combining 5-FU with platinum.

5.2. Determined mutational frequencies of the EGFR signalling pathway genes highlighted a clear similarity between the mutational patterns of UrC with CRC and bladder primary ADC, while showed a characteristic difference to that of urothelial bladder cancer.

5.3. Based on the histological and genetic similarities between UrC and CRC, therapy modalities used in CRC – such as EGFR-inhibitors – may be effective also in UrC. Targeted therapies hold promise for the treatment of rare tumors including UrC especially in those cases when chemotherapy is not effective. We found frequently occurring mutations with therapeutic relevance in the EGFR pathway in UrC. In this context, our results underline the importance of the mutation analysis of EGFR-pathway when considering an anti-EGFR therapy for a UrC patient.

5.4. Our results revealed different expressions of RHAMM, IMP3, Ki67 and p53 in UrC compared to normal tissues suggesting a role for these proteins in the formation of UrC. However, none of these differentially expressed gained a prognostic value.

6. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

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