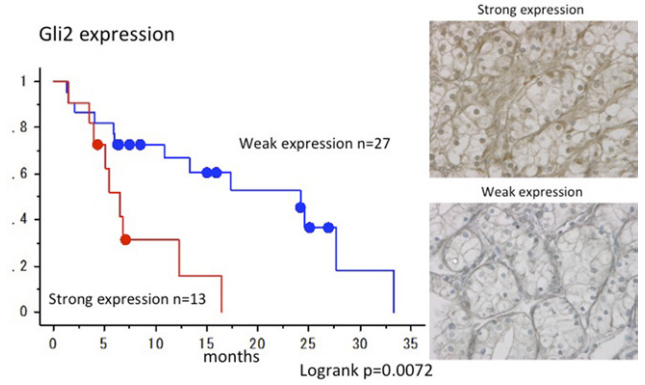
nuclear effector of hedgehog signaling. Gli2 in carcinogenesis have been shown in several human cancers. The objective of this study is to investigate the expression levels of Gli2 in radical nephrectomy specimens from patients with metastatic renal clear cell carcinoma (RCC) treated with sunitinib in order to identify factors predicting susceptibility to this agent.

METHODS: This study included a total of 40 consecutive patients undergoing radical nephrectomy, who were diagnosed as having metastatic RCC and subsequently treated with sunitinib. Gli1, Gli2 and major molecular targets of sunitinib such as VEGFR-1, VEGFR-2, PDGF-alpha and PDGF-beta expression level in primary RCC specimens were assessed by immunohistochemical staining.

RESULTS: The expression level of VEGFR-1, VEGFR-2, and Gli2, Memorial Sloan-Kettering Cancer Center risk classification, pretreatment serum calcium and c-reactive protein level were significantly associated with the progression-free survival (PFS) on univariate analysis. Of these significant factors, only Gli2 expression appeared to be independently related to PFS on multivariate analysis. In fact, PFS in patients with strong expression of Gli2 was significantly poor compared with that in those with weak expression of Gli2.

CONCLUSIONS: These findings suggest that it would be useful to consider expression levels of potential molecular marker, Gli2, as well as conventional clinical parameters to select metastatic RCC patients likely to benefit from treatment with sunitinib.



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467

VERIFICATION OF A FUNCTIONALIZED STRUCTURED MEDICAL WIRE FOR THE ISOLATION OF CIRCULATING TUMOR CELLS (CTC) IN PATIENTS WITH RENAL CELL CARCINOMA

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INTRODUCTION AND OBJECTIVES: The principal part of the metastatic renal cell carcinoma (RCC) is resistant to classical treatments. Established markers for this type of cancer, and optimized target therapies are missing. The significance and characterization of circulating tumor cells will provide insights into the selection of resistance mechanisms in patients undergoing systemic therapies. The aim of the proof of concept was to detect circulating tumor cells in metastatic RCC patients with a functionalized and structured medical wire (FSMW). The FSMW is for the in vivo isolation of CTC directly from the blood of cancer patients suitable. In clinical studies, the medical device was used in following groups of patients with non-small cell lung cancer, breast cancer and prostate cancer for the in vivo isolation of CTC.

METHODS: In the beginning, cell surface marker of renal cancer cells, such as Epithelial Cell Adhesion Molecule (EpCAM) and Mucin-1 (MUC-1) were evaluated. In this context we characterized 13 primary renal cancer cells and frozen sections of renal tumor samples with immunofluorescence analysis. In a flow system, blood samples from healthy donors were spiked with primary renal cancer cells, to check the cell-binding to the wire. The next step was a pilot study to investigate blood samples from 20 RCC patients. Additionally 5 blood samples were parallel tested in the flow system with an anti-EpCAM functionalized and anti-EpCAM/MUC functionalized wire. To confirm the CTC-binding to the wire, the immunocytochemical staining for EpCAM and Cytokeration as well as CD45 for negative cell selection was performed.

RESULTS: The primary culture cells originate from G1 and G2 RCC tumors, showed 30-100% EpCAM-expression. The MUC-1 expression is very heterogeneous in frozen sections, but increased in comparison to normal tissue. The spiking experiments indicated, that a sensitive isolation of EpCAM-positive RCC cells is possible by using the flow system. In the pilot study was the sensitivity for CTC detection 91.6 %. In the blood of patients with a local tumor on average (range) 5.3 (0-11) CTCs and in patients with a metastatic tumor average (range) 7.5 (2-24) CTC in 7.5 mL of blood were detected. EpCAM functionalized FSMW captured in mean of 8 (2-15) cells and the EPCAM / MUC-1 functionalized FSMW 6.2 (4-8) CTCs.

CONCLUSIONS: The Isolation of CTC, using the FSMW from the blood of renal cancer patients of different stages is high efficient. The EpCAM-FSMW captured more CTCs as the EpCAM/MUC-FSMW although the expression of EpCAM in renal cancer not always demonstrated.

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468

HOW DO GENETIC POLYMORPHISMS INFLUENCE IN SUNITINIB TREATED METASTATIC RENAL CARCINOMA?: A PROSPECTIVE OBSERVATIONAL STUDY AND VALIDATION.

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INTRODUCTION AND OBJECTIVES: Sunitinib (SU) is an oral, small-molecule, multi-targeted receptor tyrosine kinase inhibitor for treatment of renal cell carcinoma (RCC). In a previous study we identified a group of single nucleotide polymorphisms (SNPs) associated with survival and toxicity of RCC patients treated with SU, using a commercially available DNA microarray genotyping system. In this study, we validated our previous data using an independent series (García -Donas J, et al. Lancet Oncol 2011).

METHODS: 27 metastatic RCC patients, from January 2010 to May 2011, were evaluated prospectively. All the patients received SU standard treatment. A total of 92 of single nucleotide polymorphisms (SNPs) in 34 genes involved in the pharmacokinetic and pharmacodynamic pathways of drugs were analyzed using Drug-in-Code® pharmacogenetic service. For validation of identified polymorphism individual SNPs were performed in 83 samples using the FlexiGene DNA kit (Qiagen, USA), genotyped SNPs with the KASPar SNP genotyping system (Kbiosciences, UK) and the sequence Detection System 7900HT (Applies Biosystems, USA).

RESULTS: Patients with CYP1A2 and CYP2C19 SNPs, no statistically significant associations were observed among drug metabolizing genes and progression-free survival (PFS) or overall survival (OS). Val(158)Met Catechol-O-methyltransferase (COMT) SNPs have been associated with PFS and OS. Our study showed low metabolizing alleles (Met/Met and Val/Met) had statistical significance with PFS and OS ($p=0.0001$, $p=0.0001$) compared to Val/Val carrier. In the validation cohort, low metabolizing allele (Met/Met and Val/Met carriers) had statistical significance with PFS ($p= 0.0102$) compared to Val/Val carrier.

CONCLUSIONS: Our preliminary analysis suggests that we have found a strong association between COMT polymorphisms and