Short Communication

DETERMINATION OF K-RAS MUTATION IN COLORECTAL AND LUNG CANCER

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ABSTRACT

A mutated K-RAS gene is the originator of several characters of colorectal cancer among them tumor initiation, growth, survival, metastasis formation and even immune response. Thus; the aim of our experiments is to establish if the tumor samples express the K-RAS mutation before starting the monoclonal antibody-based therapy. Otherwise it makes no sense to block the receptor binding site if the signaling cascade is independent of the binding of the ligand to the receptor. Colorectal cancer, a cancer resulting from uncontrolled cell growth in the colon or rectum is the fourth most common cause of cancer death. The stage of its development affects the method of treatment of this disease. A prominent method to treat this type of cancer is the blockage of the EGFR by monoclonal antibodies. This kind of approach however seems only to be efficient as long as there is no mutation of K-Ras, a GTPase thought to play an important role at an early stage of the development of colorectal cancer. In case of a mutation in the RAS protein, the Ras GTPase is activated to such an extent that the subsequent over activation of downstream signaling pathways leads to tumor genesis.

***Key words*:** K-RAS, Mutation, Tumorigenesis, EGFR, Metastasis, Monoclonal Antibody

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INTRODUCTION

Colon cancer is one of the most common cancers besides breast and lung cancer. In the treatment of metastatic colorectal cancer one of the most significant therapeutic advances in cancer treatment has occurred. It will become more important in the future to look on an individual tumor's genetic profile rather than treating a specific tumor type (Astin M & Griffin, T, 2011). One particularly interesting biomarker is the epidermal growth factor receptor (EGFR), which's treatment, is very efficacious. However, patients with a tumor positive K-Ras mutation show resistance to EGFR blocking. It has been reported that approximately 30 to 50% of colon cancer patients have K-Ras mutations. This mutation in the Ras-oncogene lead to over activation of downstream signaling which in turn leads to cell transformation and tumorigenesis. Activated K-Ras not only promotes tumor initiation but also tumor growth, survival, progression, local invasion, metastasis formation, angiogenesis, and even immune response (Curr Med Chem 2006). In 2000, a study in the US showed that 32% of the assessed K-Ras mutations in colon cancer tumors showed mutations in either codon 12 or 13. Codon 12 mutations were

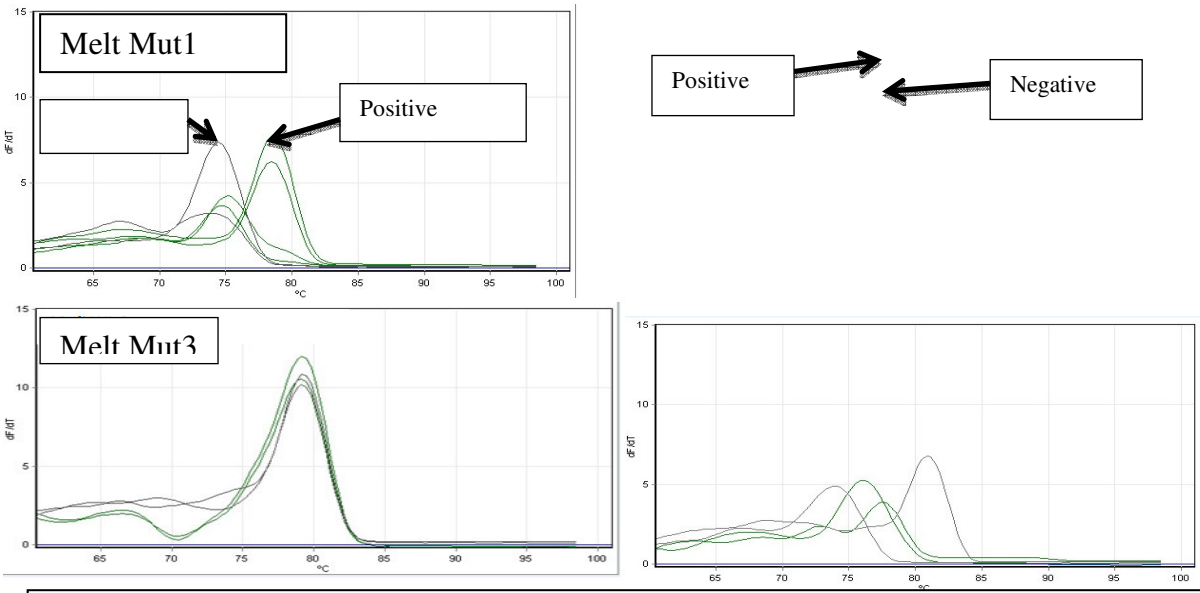
associated with advanced stage of colon cancer and a more aggressive tumor. Patients with the specific mutation glycine to valine had a significant poorer prognosis for survival. Also the codon 13 mutation showed a 40% increase in short-term mortality. It is therefore of indispensable importance to check for mutations of the K-Ras gene to see if an EGFR treatment will be successful or not. To analyze possible mutations, ARMS-PCR can be used which is described in the result section (Clin Ther, 2008).

METHODOLOGY

Immunohistochemistry staining (IHC) was the second method of choice to observe cell aggregation, invasiveness, hyper-proliferation and other cancer hallmarks. An anti-E-cadherin-antibody was used to stain E-cadherin which is mainly found on the cell membrane of the intestinal villi. As E-cadherin is thus used as a marker for the cell membrane, disordered or aggregated tumors can be seen easily through fluorescence microscopy. E-cadherin expression is often down-regulated in highly invasive, poorly differentiated tumors. The loss of expression of E-cadherin appears to be an important step in tumorigenic progression. The IHC is used in this protocol to see the tumor progression and invasiveness of the colon cancer patients (Yamanaka Y, Friess H; *Anticancer Res* 1993).

RESULTS AND DISCUSSION

Melt WTassay: The assay did not work as expected. The negative and positive controls are on the same level. A possible cause may be the cDNA synthesis did not work.



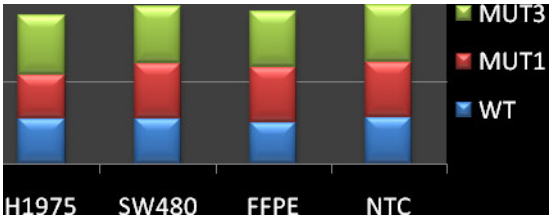
cDNA

H1975: **WT** and **Mut1** almost equal: possible case of Heterozygosity **WT/MUT1**.Booth allele are expressed on the mRNA level.

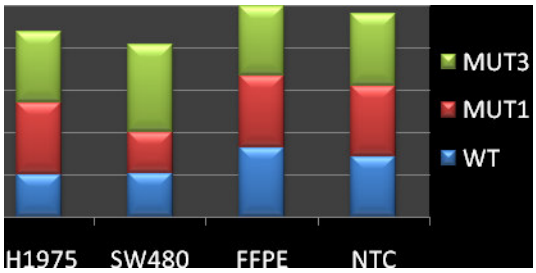
SW480: WT

FFPE: WT cT is low. This doesn´t mean that there is no another type of mutation then MUT1 and MUT3.

cDNA	H1975	SW480	FFPE	NTC
WT	27,37	27,42	25,05	28,5
MUT1	27,2	33,87	33,9	33,46
MUT3	35,94	34,34	33,29	34,18
	WT/MUT1	**WT**	**WT/??**	



Graph 1: Comparison of the cDNA expression levels of Wt, Mut1 and Mut3



Genomic DNA

H1975: WT

SW480: WT and **Mut1** almost equal: possible case of Heterozygosity **WT/MUT1**.Booth allele are present on the genomic DNA level.

FFPE: It is possible that there is another type of mutation

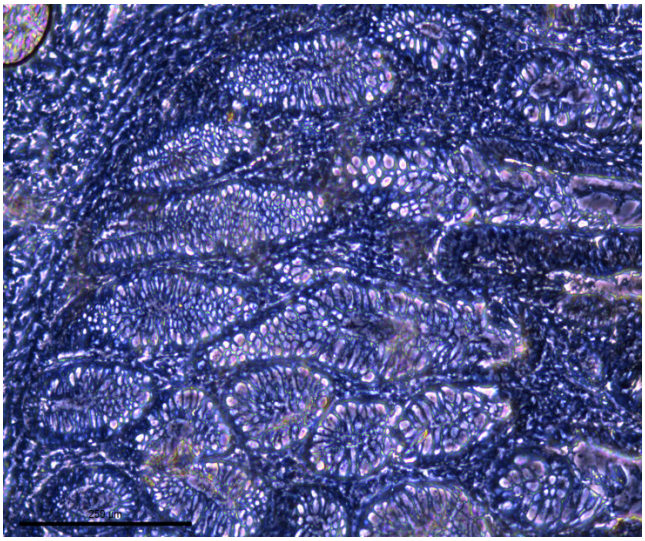
Table 2: Ct values of the gDNA

gDNA	H1975	SW480	FFPE	NTC
WT	20,3	20,77	33,12	28,5
MUT1	33,7	19,64	33,53	33,46
MUT3	33,2	40,81	32,8	34,18
	WT	**WT/MUT1**		

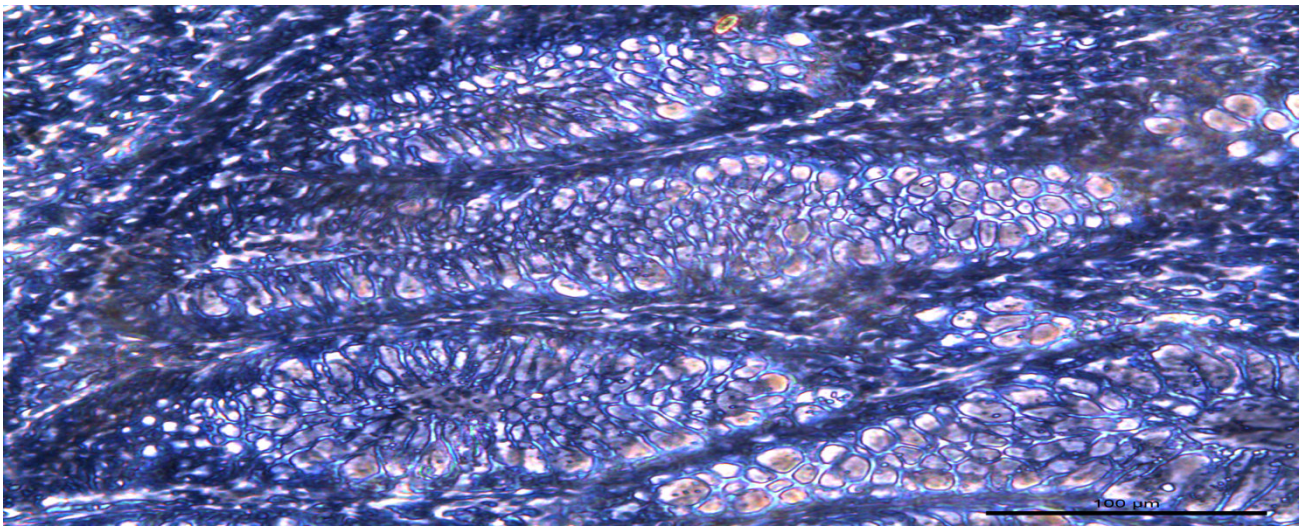
Immunohistochemistry

The immunohistochemistry assay was not successful in our case. The assay for the **wild type** didn't work. A possible cause is that the cDNA synthesis did not work. This affects all the other results. But supposing that the assay worked, we can say that the most mutation observed in the samples is MUT1 and it is observed in a heterozygotic state (Mut1/Wt) (Nat Clin Pract Oncol, Clin Ther, 2008)

IHC: It is also very important to determine if the E-cadherin have been down regulated (Cadherin switch) to be able to make a prognosis. Most of the cases an E cadherin down regulation is sign of an invasive behavior of the tumor. In this case the surgical treatment is not a good alternative and the chemotherapy seems to be more appropriate (Astsaturov I, Cohen RB, Harari P*; Expert Rev Anticancer Ther* 2006).



The results of the ICH assay were not satisfactory. Many causes can lead to that. Example of possible cause:
*Dehydration steps may be not long enough (time)
*Incubation time with the antibodies
*Non-reactive tissue can lead to lack of staining
* The antibody itself can be deteriorated or not adequately used and conserved (repeated thawing freezing cycles)



CONCLUSION

The determination of K- Ras mutation in the colorectal cancer cells is a pre-requisite before starting any monoclonal antibody-based therapy (Klein S and Levitzki A, 2009). Many mutations can affect the signaling of K- Ras and thus the samples should be tested for all of the known mutations but at least the most frequent ones.

ACKNOWLEDGEMENTS

We thank Dr. Elisabeth Hofmann for technical assistance and Dr. Eger helped for qPCR, ARM-PCR, as well as statistical assistance .The isolated cancerous tissue used for this study was kindly given by miss Tina IMC Krems, Krems Austria.

This study work was supported by Grant gratefully given by Department of Medical Biotechnology, IMC University of applied sciences Krems, Austria.

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