

# The Association between Mutations Detected in Tissue and Plasma from Patients with Colorectal Adenoma and Adenocarcinoma

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## Abstract

Most colorectal cancers evolve through an adenoma-carcinoma sequence with mutations in the KRAS/BRAF pathway as an early event. Mutation analyses are usually performed on tissue samples, but during the last couple of years the same analysis in blood has been facilitated. Our aim was to investigate the correlation between BRAF/KRAS mutations in tissue and plasma from colorectal adenomas and adenocarcinomas. Out of 22 patients with adenomas 10 had a mutation in the tissue, but no mutations were detected in the plasma. In 10 of 26 adenocarcinomas a mutation was found in the tumor and in four of these, the mutation was also detected in the plasma. Our results confirm previous findings that mutated DNA in plasma can be detected in approximately 50% of non-metastasized adenocarcinomas. The difference between adenomas and adenocarcinomas suggests that appearance of mutated DNA in plasma associates with invasion.

## Keywords

KRAS, BRAF, Adenomas, Adenocarcinomas, Carcinogenic Development

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## 1. Introduction

Colorectal cancer (CRC) is a common disease worldwide with high morbidity and mortality. Most cases evolve through an adenoma-carcinoma sequence [1] with few and unspecific symptoms. Adenomas are typically classified according to their histology as tubular, tubulovillous, villous or serrated. Early and timely detection of malignant progression is crucial to curative treatment [2].

Mutation analyses are usually performed in formalin fixed, paraffin embedded tissue samples, but in recent years it has become possible to detect tumor DNA in plasma [3]. KRAS mutations occur early in the carcinogenesis and are recognized to play an important role in progression from adenoma to carcinoma. RAS proteins participate in the RAS-RAF-MEK-ERK-MAP kinase pathway and thereby play a role in a wide variety of cellular functions such as cell proliferation and apoptosis [4]. The size of the adenoma is important, since small adenomas are less likely to have a RAS mutation [5]. BRAF mutations are found in approximately 10% of CRCs.

The aim of the present study was to investigate the correlation between BRAF/KRAS mutations in tissue and plasma of adenomas and adenocarcinomas (ACs) to determine whether the carcinogenic development was reflected in plasma.

## 2. Material and Methods

Representative blocks of 48 colorectal adenomas and colorectal ACs from patients treated at the Department of Surgical Gastroenterology, Copenhagen University Hospital, Hvidovre, Denmark, were retrieved from the archive. All lesions were localized without distant metastasis. The tissue was formalin fixed and paraffin embedded. The cancers were classified according to the TNM system, and immunohistochemistry for mismatch repair proteins was performed. Blood samples obtained at the time of diagnosis were centrifuged at  $2.500 \times g$  for 10 minutes at room temperature and plasma samples were immediately frozen and stored at  $-80^{\circ}\text{C}$  under constant electronic surveillance.

For the tumor mutation analysis, DNA was purified from three 10  $\mu\text{m}$  sections of FFPE tissue using a Maxwell 16 FFPE Tissue LEV DNA purification kit on a Maxwell 16 Instrument (Promega, WI, USA) according to the manufacturer's instructions. DNA was analyzed with in-house ARMS-qPCR assays [6]. The assays detect the seven most common KRAS mutations and the BRAF V600E mutation.

The plasma DNA mutation analysis was based on 4 ml of plasma purified on a Qiasymphony purification system using a Qiasymphony DSP Virus/Pathogen midi kit (Qiagen, Hilden, Germany). DNA was pre-amplified in a 50  $\mu\text{l}$  reaction using  $2\times$  TaqMan PreAmp Master Mix (Thermo Fisher, MA, USA) and a pre-amplification primer mix consisting of primers amplifying a 94 bp fragment of the KRAS gene and a 120 bp fragment of the BRAF gene. The pre-amplification conditions were 10 min at  $95^{\circ}\text{C}$ , 2 min at  $55^{\circ}\text{C}$  and 2 min at  $72^{\circ}\text{C}$  followed by 10 cycles of  $95^{\circ}\text{C}$  for 15 sec and  $60^{\circ}\text{C}$  for 4 min. The final inactivation step was  $99.9^{\circ}\text{C}$  for 10 min. The pre-amplified DNA was diluted 50 times in TE buffer and analyzed with the in-house KRAS and BRAF assays.

## 3. Results

The study included 22 adenomas and 26 ACs (T1-T4). In colorectal adenomas KRAS/BRAF mutations occurred in approximately half of these pre-malignant colorectal lesions (10/22, 45%). Ten adenomas had a KRAS mutation, but none had a BRAF mutation. Mutated DNA in plasma was not detected in any of the 10 patients with KRAS mutated adenomas.

Out of the 26 ACs, six had a KRAS mutation (23%) and four a BRAF mutation (15%) in the tissue, a total of 10 mutations (38%). Out of these 10 AC with mutations in the tissue, two had KRAS and two BRAF mutations in the plasma. There was an explicit agreement between the tissue and plasma mutations.

## 4. Discussion

There are no previous studies investigating mutated DNA in plasma from patients with adenomas. We investigated the frequency of KRAS and BRAF mutations in adenomas and ACs in 48 tissue samples with corresponding blood samples and confirmed previous results that KRAS/BRAF is detectable in tissue of pre-malignant lesions and not only in ACs [7]-[9].

Previous studies have shown that KRAS/BRAF mutations are detected in plasma in approximately 50% of localized tumors, but with a higher rate in metastatic tumors [3]. Our present study adds to these results. We de-

tected KRAS/BRAF in the plasma of 40% of the patients who had these mutations in the tissue, all with localized cancer stage I-III. The transition from adenoma to an invasive tumor is expected to represent a change in the biology towards more aggressive characteristics.

The results presented here favor the hypothesis that this transition may be accompanied by a release of mutant DNA into the blood. Also, it may suggest that the occurrence of mutant DNA in plasma from invasive tumors indicates a higher degree of malignancy. Furthermore, the lack of mutated DNA in plasma from adenoma patients suggests that the biopsy procedure is not a source of tumor DNA in plasma.

## 5. Conclusion

In conclusion, our results suggest that the presence of tumor DNA in plasma is specific for invasive neoplasms. The possible biological and clinical implications remain to be elucidated and call for prospective studies of a sufficient size to confirm or discard the achieved results.

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