Human Pathology (2007) **38**, 1696–1702

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

High-degree tumor budding and podia-formation in sporadic colorectal carcinomas with K-ras gene mutations

Friedrich Prall MD*, **Christiane Ostwald PhD**

Institute of Pathology, University of Rostock, D-18055 Rostock, Germany

Received 27 January 2007; revised 30 March 2007; accepted 3 April 2007

Keywords:
Colorectal carcinoma;
Tumor budding;
Epithelial-mesenchymal transition;
K-ras mutation

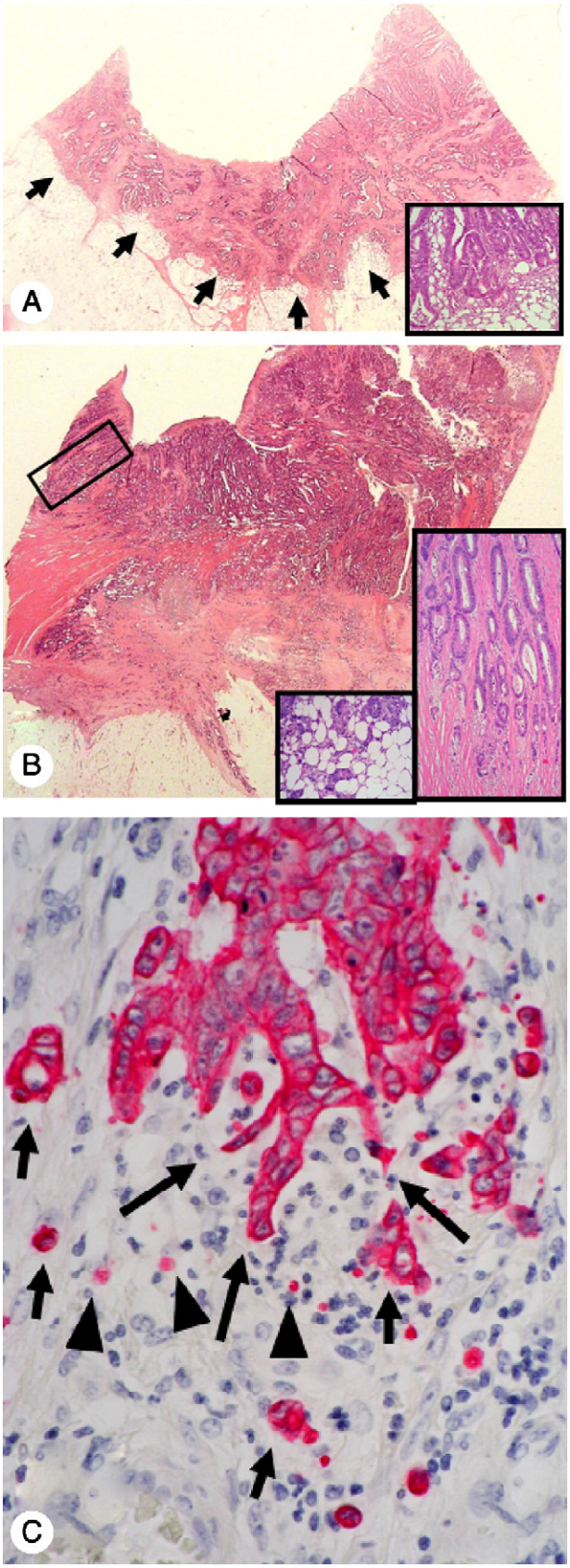
Summary In vitro ras activation enhances the epithelial-mesenchymal transition of colorectal carcinoma cells. But ras effects are known to be highly dependant on cell types and the tissue context. Therefore, this study was made to test the hypothesis that in clinical colorectal carcinoma specimens, aggressive invasion phenotypes, specifically tumor budding and podia formation, would correlate with K-ras gene mutations. In a series of 95 clinically sporadic primary colorectal carcinomas collected ad hoc, tumor budding and podia formation were counted using pan-cytokeratin immunohistochemistry, and K-ras gene mutations in codons 12 and 13 were determined. Consistent with the hypothesis, tumor budding and podia formation were observed to be significantly higher in the 32 (34.7%) of the tumors with K-ras gene mutations (29 mutations in codon 12, 3 in codon 13), and this correlation was observed independent of the patterns of invasion (expansive versus infiltrative). Microsatellite status, numbers of losses of heterozygosity, adenomatous polyposis coli and p53 gene mutations, and degree of promoter methylations (CIMP status) were not associated with K-ras gene mutations. Besides their effects on the tumor cell cycles, oncogeneic K-ras gene mutations in colorectal carcinomas could be important for aggressive tumor invasion. This may be important in metastasizing disease and could provide a rationale for developing drugs that interrupt ras-signaling cascades.

1. Introduction

As tumor cells interact with the surrounding stromal cells and the extracellular matrix, tumor invasion in colorectal adenocarcinomas is a highly complex process. At the tumor-host interface, the extracellular matrix is continually broken down by proteolysis on one hand, and new extracellular matrix is synthesized and laid down on the other hand (desmoplasia). Furthermore, tumor cells, singly or in larger aggregates, can migrate into the desmoplastic extracellular matrix. If studied histomorphologically in clinical specimens with attention to detail, tumor invasion in colorectal carcinomas is seen to differ considerably between cases, and a classification of colorectal carcinomas according to different invasion phenotypes can be made. Specifically, there can be observed the expansive versus the infiltrative invasion pattern [1] as well as different degrees of tumor budding [2], and podia formation [3] (reviewed in Prall [4]; examples in Fig. 1). Importantly, as tumor invasion is a first step in the metastatic cascade, these different invasion phenotypes are closely correlated to differences in clinical aggressiveness [1,2,5,6].

* Corresponding author.
E-mail address: friedrich.prall@med.uni-rostock.de (F. Prall).

0046-8177/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2007.04.002



Cellular biological and molecular mechanisms of tumor invasion have been under intensive investigation in experimental and in vitro systems, and features analogous to the different invasion phenotypes are reported under designations as epithelial-mesenchymal transition (EMT), cohort migration, or mesenchymal-amoeboid transformation (reviewed in Friedl and Wolf [7]). Many different epigenetic factors are known to influence invasiveness, for example, matrix degrading enzymes, matrix components, and growth factors. However, genetic factors inherent to the tumor cells, too, were seen to play a role. Specifically, in colorectal carcinoma cells, ras-signaling was shown to be important for the EMT [8,9].

Normal, nonmutated Ras proteins are activated in response to growth factors binding to membranous receptor tyrosine kinases. In a signaling cascade involving phosphorylation of extracellular mitogen-activated kinases (ERKs), the signal is relayed to the nucleus where it activates transcription factors that influence cell proliferation control (eg, c-Jun, c-Myc, c-Fos). Alternatively, activated Ras through phosphoinositide 3-kinase acts on actin filaments within cells. Accordingly, processes as diverse as cell proliferation and modulation of cell shape and cell migration are stimulated by ras-signaling. These cellular biological effects of ras activation heavily depend on cell types and the tissue context (reviewed in Malliri and Collard [10]).

In colorectal carcinomas, the K-ras gene is known to contain activating (ie, oncogeneic) point mutations in about 30% of the tumors [11,12]. In this study, using clinical specimens of colorectal carcinomas, we tested the hypothesis derived from in vitro observations that K-ras mutation has an effect on invasion phenotypes. Specifically, in a series of clinically sporadic colorectal carcinomas, types of tumor invasion and quantified data of tumor budding as well as podia formation were related to the K-ras mutational status.

Fig. 1 A, Colorectal carcinoma with an expansive pattern of tumor infiltration. Note the branching neoplastic glands and the sharply demarcated deep invasive margin (arrows) (detail in the inset to the right). Whole-mount section. B, In contrast, colorectal carcinomas with an infiltrative pattern of growth dissect the tunica muscularis with long-stretched neoplastic glands (boxed area) (higher magnification in the inset to the right); in the extramural adipose tissue, neoplastic glands dissolve into loosely arranged microglandular proliferations (higher magnification in the inset to the left). Overall, the invasive margin is ill-defined. Whole-mount section. C, Example of tumor budding and podia formation as seen on pan-cytokeratin immunostains. From the neoplastic glands at the invasive margin, small strands of tumor cells extend into the stroma (long arrows); from these, small tumor cell complexes (tumor buds) seem to have become detached (short arrows). Tumor buds are surrounded by podia; these are observed in pan-cytokeratin immunostains as halos of very small a-nucleate globules (arrowheads) (antibody MNF116, ×40 objective).