Cysteine-rich Fibroblast Growth Factor Receptor 1, a New Marker for Precancerous Epithelial Lesions Defined by the Human Monoclonal Antibody PAM-1

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ABSTRACT

Precancerous epithelial lesions are sites of uncontrolled cellular proliferation, generated by irreversible genetic changes. Not all of these lesions progress to invasive cancer, some may even regress, but early detection of abnormal cells can be crucial for survival of the patient. Diagnosis is mainly performed by using morphological parameters. Proliferation markers can facilitate the analysis, if they show a consistent expression, and distinguish between healthy and malignant cells. The fully human monoclonal IgM antibody PAM-1 was isolated from a patient with stomach carcinoma and binds to a new variant of cysteine-rich fibroblast growth factor receptor 1 (CFR-1). This CFR-1/PAM-1 receptor is expressed on nearly all of the epithelial cancers of every type and origin, but not on healthy tissue. It is also present on precursor lesions found in: Helicobacter pylori-induced gastritis, intestinal metaplasia and dysplasia of the stomach, ulcerative colitis-related dysplasia and adenomas of the colon, Barrett’s metaplasia and dysplasia of the esophagus, squamous cell metaplasia and dysplasia of the lung, and cervical intraepithelial neoplasia. The unique, growth-dependent expression of this new CFR-1 isoform makes the PAM-1 antibody an ideal diagnostic tool for the detection of precancerous and cancerous lesions.

INTRODUCTION

A multicellular organism is a perfectly organized compound of differentiated cells and organs. All of the cellular processes, like proliferation, regeneration, repair, and so forth, are under strict control. However, with billions of cell divisions during a single lifetime, natural and induced mutagenesis occur frequently, and can lead to serious genetic changes and aberrant growth of cells. Nearly all of these abnormal cells are detected at a very early stage by host control mechanisms, but in very rare cases some cells can escape these controls and become tumor precursor lesions. These tumor precursor lesions can, in turn, develop into tumors leading to damage and possibly death of the host.

Tumor prevention, in addition to the identification of risk factors, is based mainly on the detection of cancer precursor lesions with the chance of most solid cancers being successfully treated if diagnosed early. The detection of these precancerous cells is based on a microscopic analysis of biopsy material. This morphological diagnosis, the differentiation between healthy and malignant tissue, is in general difficult to perform, because the cellular changes are often minimal. Standard histological methods are also hampered by the fact that analysis is influenced by the subjectivity of the observer (1). Therefore, additional immunohistochemical methods are helpful for the detection of cellular malignant changes, e.g., the use of proliferation markers. However, most proliferation markers, like Ki67, do not differentiate between healthy and malignant cells, and neither do they react with all tumor cells (2–4), which makes clear grading sometimes difficult.

The best source for tumor-specific diagnostic tools is the antibody pool of the individual cancer patient. Tumor-specific antibodies can be detected in sera (Serex; Refs. 5, 6) or can be established by using hybridoma technology to immortalize lymphocytes (7–11).

Conventional human hybridoma technology by immortalizing lymphocytes from cancer patients offers the unique possibility to generate new fully human monoclonal antibodies for the diagnosis and therapy of diseases, and, in addition, to characterize new tumor-related membrane receptors with a single experimental approach (9, 12–16). This is not possible with any other conventional hybridoma technology or monoclonal antibody engineering technology (phage display).

Using this human hybridoma technology we have isolated and described previously the human antibody SC-1, generated from a patient with a signet-ring-cell carcinoma of the stomach (7). The receptor for SC-1 was found to be a modified version of CD55 (decay-accelerating factor). Stomach carcinoma cells express two different forms of CD55/decay-accelerating factor on the cell surface: (a) the normal $M_r$ 70,000 isofrom that protects against complement attack; and (b) the CD55/SC-1 apoptosis receptor described recently. The CD55/SC-1 isofrom is overexpressed on gastric carcinoma cells (13) and has a molecular weight of $M_r \sim 82,000$. The IgM antibody SC-1 induces apoptosis of gastric cancer cells in vitro and in vivo, and is being used successfully in clinical trials (12, 13, 15–17).

The fully human germ-line coded monoclonal IgM antibody PAM-1 (clone 103/51) was also isolated from a patient with a stomach carcinoma. PAM-1 reacts with a membrane receptor present on nearly all of the epithelial cancers of every type and origin. When evaluated on nonmalignant tissue, the only PAM-1-specific reactivity was an intracellular binding to proteins in Golgi apparatus of the kidney (14). The receptor for PAM-1 was purified from tumor cell membrane extracts and was found to be a $M_r$ 130,000 integral membrane glycoprotein (14), homologous to CFR-1,2 which has thus far only been detected and described in Golgi of embryonic chicken cells and in Chinese hamster ovary cells (18). The receptor is homologous to a rat protein, cloned as a Golgi-specific protein, designated MG160, which is involved in the processing and secretion of growth factors and was found recently in pancreatic cancer (19–23). The human homologue, E-selectin ligand 1, is a cytokine, expressed on myeloid and some lymphoma cells, and is modulated by cell adhesion molecules that cause the binding of neutrophils to the endothelium (24, 25). In this study we show that CFR-1, detected by the human antibody PAM-1 is, in contrast with other proliferation markers, a reliable target for the detection of precancerous and cancerous lesions.

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2 The abbreviations used are: CFR-1, cysteine-rich fibroblast growth factor receptor 1; CIN, cervical intraepithelial neoplasia; PCNA, proliferating cell nuclear antigen; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
MATERIALS AND METHODS

Cell Culture. The human hybridoma cell line 103/51 (PAM-1) was produced and grown as described previously (9).

RNA Isolation. RNA from normal and cancerous gastric tissue of the stomach was isolated using the phenol-guanidine-isothiocyanate method with TRIzol reagent (Invitrogen). In brief, frozen normal and tumor tissues were cut in serial 5-μm sections on a freezing microtome. One ml of TRIzol reagent was added to the tissue samples, and the solutions were homogenized subsequently. After homogenization the insoluble material was removed from the homogenate by centrifugation at 12,000 × g for 10 min at 4°C. Two-hundred μl of chloroform was added to the RNA containing supernatant, and after mixing, the solution was incubated for 3 min at room temperature. After centrifugation for 15 min at 12,000 × g and 4°C, the aqueous phase was precipitated in 500 μl of isopropanol by mixing for 30 s, incubation for 10 min at room temperature, and centrifugation for 10 min at 12,000 × g and 4°C. The resulting RNA pellet was washed with 1 ml of 75% ethanol and centrifuged for 5 min at 7,500 × g at 4°C. The RNA pellet was air-dried and resuspended in 80 μl diethyl pyrocarbonate-treated water. The integrity and quality of purified total RNA were controlled by 1% agarose gel electrophoresis, and the concentrations were evaluated by spectrophotometry.

Semiquantitative RT-PCR. mRNA levels were examined using semiquantitative RT-PCR method. Synthesis of first-strand cDNA from normal and cancerous gastric tissue was performed with 5 μg of total RNA using Moloney murine leukemia virus reverse transcriptase (Invitrogen GmbH, Karlsruhe, Germany) and oligodeoxythymidylic acid primer according to the suppliers manual. The PCR method was used to detect CFR-1 mRNA. PCRs were carried out in a 25-μl volume with 2 mM MgCl₂, 0.4 μM primer, 200 μM each deoxynucleoside triphosphate, and 1 unit of Taq polymerase (MBI). The expression of CFR-1 mRNA was normalized to GAPDH mRNA levels. The primers specific for CFR-1 and GAPDH were designed on their reported sequences and commercially synthesized by MWG-BIOTECH AG (Ebersberg, Germany). The sequences of these oligonucleotides are 5’ CAAGAGCAGACTGAGTGG 3’ and 5’ CCGGAAGTTGTTGAGG 3’ for CFR-1, and 5’ GTGGAGGACTCATGACCAGTC 3’ and 5’ CATGTT-
GGGCCATGAGGTCCACCAC 3′ for GAPDH. Sizes of expected amplification products are 750 bp for CFR-1 and 482 bp for GAPDH. CFR-1 was amplified at 94°C for 4 min and for 40 cycles at 94°C (30 s), 55°C (30 s), and 72°C (30 s) with a final extension step at 72°C (4 min). As a negative control each PCR run included a sample containing PCR buffer but no cDNA. The PCR products were identified by agarose-gel electrophoresis (2%) in Tris-acetate-EDTA buffer and ethidium bromide staining.

Immunohistochemical Staining of Paraffin Sections. Paraffin-embedded human tissues were sectioned (2 μm), deparaffinized, and heated in citric acid (pH 5.5) in a pressure cooker for 5 min. Then the sections were blocked with BSA (5 mg/ml) diluted in PBS for 30 min at room temperature. The treated sections were then incubated either with PAM-1 (10 μg/ml) or anti Ki67 antibody (Loxo, Dossenheim, Germany; diluted 1:20 with BSA/PBS) for 2.5 h at 37°C in a humidified incubator. After incubation the sections were washed three times with Tris/NaCl, followed by incubation with peroxidase-labeled rabbit antihuman or rabbit antimouse conjugate (Dako, Hamburg, Germany) diluted 1:50 in PBS containing rabbit serum (for antibody PAM-1) for 1 h at room temperature. After washing three times with Tris/NaCl and incubation in PBS for 10 min staining was performed with diaminobenzidine (0.05%) -hydrogen peroxide (0.02%) for 10 min at room temperature. The reaction was stopped under running tap water, and sections were counterstained with hematoxylin. After mounting with glycerol gelatin, the sections were analyzed using light microscopy.

RESULTS

Expression of CFR-1/PAM-1 on Normal Tissue. The CFR-1/PAM-1 receptor defined by the human monoclonal antibody PAM-1 is a new membrane-bound proliferation marker, specifically expressed on malignant, but absent on nontransformed tissue. This has been shown in detail in a previous publication (14). However, to confirm and to illustrate the absence of the CFR-1/PAM-1 receptor from normal tissue, selected paraffin tissue sections from different normal tissues have been stained with antibody PAM-1. On normal tissue sections of the stomach, the uterus, the thyroid gland, and the spleen (Fig. 1, A–D), no recognizable expression of CFR-1/PAM-1 can be found. These data demonstrate that the CFR-1/PAM-1 receptor is really absent from nonmalignant tissue.

Fig. 2. Immunohistochemical staining of PAM-1 on different carcinomas. Paraffin sections were stained with H&E, antibody Ki67, and antibody PAM-1: A, invasive lobular carcinoma of the breast; B, adenocarcinoma of the cardia; C, esophageal squamous cell carcinoma; D, adenocarcinoma of the prostate. Original magnification. ×100.
Carcinoma of the breast (Fig. 2) of this protein remains unknown (26), but it is the most widely used marker on malignant tissue, 5–8 and to illustrate the highly specific expression of CFR-1/PAM-1 on adenomas. The expression of CFR-1 could be detected in gastric tumor tissue compared with normal gastric tissue (Fig. 3). This shows that CFR-1 detected by antibody PAM-1 on malignant cells is a specific overexpressed and most likely post-transcriptionally modified isoform of CFR-1.

Expression of CFR-1/PAM-1 on Premalignant Tissue. CFR-1/PAM-1 is expressed in stomach cancer precursor lesions like H. pylori-induced gastritis and gastric dysplasia, and there is evidence that the level of expression increases with the escalation of malignancy (14). To manifest and improve this observation and for a comparison with the proliferation marker Ki67, additional immunohistochemical experiments were performed with a variety of other precursor lesions. In the following chapters, illustrations of precursor lesions from colon, esophagus, cervix, and bronchial carcinoma, and corresponding proliferation zones are shown to demonstrate the specific expression of PAM-1. The data are summarized in Table 2.

Adenoma-Carcinoma Sequence. Malignant changes and the resulting carcinomas of the colon belong to the frequently occurring neoplasias and are often associated with high mortality. The formation of colon carcinomas is a multistep process, which could be retraced to the so-called adenoma-carcinoma sequence. All of the adenomatous lesions arise as a result of epithelial proliferative changes, and there is strong evidence that adenomas are precursor lesions for invasive colorectal adenocarcinoma (27–29). As such, colorectal carcinogenesis provides the ideal opportunity to investigate the reactive pattern of PAM-1 in preneoplastic lesions in more detail. Therefore, additional immunohistochemical stainings were performed on different types of mucosa and epithelia. As reported earlier, PAM-1 antibody reacts with H. pylori-associated chronic active gastritis, high-grade dysplasia, and gastric adenocarcinoma (14). This study not only confirmed the results of the earlier report but enhanced the available data by also including atrophic gastritis and intestinal metaplasia, which are preneoplastic stages in the gastric carcinogenesis. A positive PAM-1 staining was observed on both preneoplastic lesions (data not shown).

Therefore, the reaction of PAM-1 on colon mucosa, tubular and villous adenomas, ulcerative colitis-related dysplasia, and adenocarcinomas of the colon was examined (Fig. 4).

Noninflamed colon mucosa showed no reaction (Fig. 4A). An increasing expression of CFR-1/PAM-1 was found in adenomas of the colon (Fig. 4B), which have a higher risk of degenerating into adenocarcinomas. Expression of CFR-1/PAM-1 was seen both in tubular and in villous adenomas, particularly in the proliferation zone.

Expression of CFR-1/PAM-1 on Malignant Tissue. To study and to illustrate the highly specific expression of CFR-1/PAM-1 on malignant tissue, 5–13 different cases of the most frequently occurring carcinomas were tested immunohistochemically. The reactivity of PAM-1 was compared with the expression of the Ki67 protein, which is localized in the nucleus of all of the proliferating cells. The function of this protein remains unknown (26), but it is the most widely used standard marker for proliferation studies (2, 3).

The staining results are exemplary shown on invasive lobular carcinoma of the breast (Fig. 2A), adenocarcinoma of thecardia (Fig. 2B), esophageal squamous cell carcinoma (Fig. 2C), and adenocarcinoma of the prostate (Fig. 2D), and summarized in Table 1. Taken together, whereas PAM-1 shows a broad, intensive, and homogeneous staining on all of the carcinomas, Ki67 is not found in all of the carcinomas; it shows only a weak expression in most cases and in contrast to PAM-1, it is inhomogeneous distributed (Table 1; Fig. 2). Adenocarcinomas of the liver (hepatocellular carcinoma) are all negative, and only several cases of adenocarcinomas of prostate, lung, and invasive lobular carcinomas of the breast are positive for Ki67. Assuming that tumor cells are always highly proliferative, our data might indicate that the membrane-bound CFR-1/PAM-1 receptor is a more reliable marker for malignancy than the Ki67 protein. These data also strongly confirm what has already been mentioned in a previous study (14), namely that CFR-1/PAM-1 is expressed specifically on most tested carcinomas.

Gene Expression of CFR-1 in Normal and Tumor Tissue. PAM-1 antibody reacts with a N-linked carbohydrate residue on CFR-1/PAM-1, found specifically on malignant cells (14). Therefore, the lack of PAM-1 binding to nonmalignant cells can be either the result of a nonexpression of the receptor on normal cells or of a post-transcriptional modification of CFR-1, which is different to that found on malignant cells. We investigated this on molecular level. CFR-1 mRNA of nonmalignant and cancerous gastric tissue was examined using semiquantitative RT-PCR. The RT-PCR method was standardized by using the constitutively expressed “housekeeping gene” GAPDH as an internal control. An increased expression level of CFR-1 could be detected in gastric tumor tissue compared with normal gastric tissue (Fig. 3). This study not only confirmed the results of the earlier report but enhanced the available data by also including atrophic gastritis and intestinal metaplasia, which are preneoplastic stages in the gastric carcinogenesis. A positive PAM-1 staining was observed on both preneoplastic lesions (data not shown).

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Table 1: Expression of CFR-1/PAM-1 on tumors tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Carcinoma type</th>
<th>Sex</th>
<th>Ki67 staining</th>
<th>PAM-1 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>Squamous cell</td>
<td>M</td>
<td>4/1</td>
<td>5/0</td>
</tr>
<tr>
<td>Stomach</td>
<td>Adeno (Barrett)</td>
<td>F</td>
<td>12/1</td>
<td>11/2</td>
</tr>
<tr>
<td>Stomach</td>
<td>Adeno (diffuse)</td>
<td>F</td>
<td>3/2</td>
<td>2/3</td>
</tr>
<tr>
<td>Stomach</td>
<td>Adeno (intestinal)</td>
<td>F</td>
<td>2/3</td>
<td>5/0</td>
</tr>
<tr>
<td>Colon</td>
<td>Adeno</td>
<td>F</td>
<td>7/1</td>
<td>50-74</td>
</tr>
<tr>
<td>Colon</td>
<td>Adeno</td>
<td>F</td>
<td>8/1</td>
<td>5-38</td>
</tr>
<tr>
<td>Liver</td>
<td>Adeno (HCC)</td>
<td>F</td>
<td>8/1</td>
<td>43-76</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Adeno (dual)</td>
<td>F</td>
<td>5/3</td>
<td>41-75</td>
</tr>
<tr>
<td>Lung</td>
<td>Adeno</td>
<td>F</td>
<td>8/3</td>
<td>42-78</td>
</tr>
<tr>
<td>Mamma</td>
<td>Invasive (dual)</td>
<td>F</td>
<td>0/5</td>
<td>37-88</td>
</tr>
<tr>
<td>Mamma</td>
<td>Invasive (lobular)</td>
<td>F</td>
<td>0/5</td>
<td>40-89</td>
</tr>
<tr>
<td>Ovary</td>
<td>Adeno</td>
<td>F</td>
<td>8/1</td>
<td>37-80</td>
</tr>
<tr>
<td>Uterus</td>
<td>Adeno</td>
<td>F</td>
<td>0/9</td>
<td>50-80</td>
</tr>
<tr>
<td>Cervix</td>
<td>Squamous cell</td>
<td>F</td>
<td>0/11</td>
<td>46-70</td>
</tr>
<tr>
<td>Prostate</td>
<td>Adeno</td>
<td>F</td>
<td>9/0</td>
<td>49-70</td>
</tr>
</tbody>
</table>

Fig. 3. Expression of CFR-1/PAM-1 on mRNA level in nonmalignant and cancerous gastric tissue (C) and gastric tumor tissue (D) and reverse transcribed. The expression of CFR-1 mRNA was normalized to GAPDH mRNA levels. As a negative control, each PCR run included a sample containing PCR buffer but no cDNA (B). A 100-bp DNA ladder was used as marker (A). PCR-amplification products were separated on a 2% agarose gel and visualized by ethidium bromide staining.

Table 2: Expression of CFR-1/PAM-1 on precancerous tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Precursor lesions</th>
<th>Sex</th>
<th>Ki67 staining</th>
<th>PAM-1 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>Barrett metaplasia</td>
<td>M</td>
<td>9/0</td>
<td>42-69</td>
</tr>
<tr>
<td>Stomach</td>
<td>H. pylori gastritis</td>
<td>F</td>
<td>5/0</td>
<td>24-86</td>
</tr>
<tr>
<td>Stomach</td>
<td>Atrophic gastritis</td>
<td>F</td>
<td>5/0</td>
<td>25-79</td>
</tr>
<tr>
<td>Stomach</td>
<td>Intestinal metaplasia</td>
<td>F</td>
<td>5/0</td>
<td>49-86</td>
</tr>
<tr>
<td>Stomach</td>
<td>Tubular adenoma</td>
<td>F</td>
<td>5/0</td>
<td>42-87</td>
</tr>
<tr>
<td>Stomach</td>
<td>Tubulovillous adenoma</td>
<td>F</td>
<td>2/0</td>
<td>54-84</td>
</tr>
<tr>
<td>Colon</td>
<td>Dysplasia (ulcerative colitis)</td>
<td>F</td>
<td>4/0</td>
<td>42-57</td>
</tr>
<tr>
<td>Colon</td>
<td>Tubular adenoma</td>
<td>F</td>
<td>5/0</td>
<td>54-85</td>
</tr>
<tr>
<td>Cervix</td>
<td>Villous adenoma</td>
<td>8/2</td>
<td>45-85</td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>High grade dysplasia</td>
<td>F</td>
<td>3/0</td>
<td>65-74</td>
</tr>
<tr>
<td>Bronchus</td>
<td>Squamous metaplasia</td>
<td>F</td>
<td>5/0</td>
<td>64-75</td>
</tr>
</tbody>
</table>

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Ulcerative colitis-related dysplasia, which consists of atypical changes in epithelial cells, is also recognized to be involved in the development of colorectal adenocarcinoma (30). On this high-grade dysplasia, clear staining for PAM-1, especially of these atypical epithelial cells, was observed (Fig. 4C).

The most intensive staining was found in colorectal adenocarcinoma (Fig. 4D) after the obtained results in case of gastric mucosa. Here, the expression of CFR-1/PAM-1 correlates with the pattern of Ki67.

**Barrett’s Carcinogenesis.** Barrett’s esophagus is a complication of long-standing gastroesophageal reflux. The distal squamous mucosa is replaced by metaplastic columnar epithelium, as a response to prolonged injury. The carcinogenesis of esophageal adenocarcinoma takes place from Barrett’s metaplasia to Barrett’s dysplasia (31–34).

Because of the increasing incidence of Barrett’s carcinoma, the expression of CFR-1 on Barrett’s epithelium was investigated using immunohistochemical staining with PAM-1 (Fig. 5). Staining with PAM-1 revealed an increasing expression of CFR-1/PAM-1 in the metaplastic columnar epithelium of Barrett’s metaplasia (Fig. 5A). In addition, an intensive staining pattern was observed in Barrett’s dysplasia (Fig. 5B), especially those cells with architectural and cytological abnormalities. The latter are regarded as precursors of the invasive adenocarcinomas of the esophagus (Barrett’s carcinoma; Ref. 34), and correlated with expression of Ki67. The strongest staining was found in Barrett’s carcinoma (Fig. 5C). Although PAM-1 showed an intensive staining pattern for carcinoma of the cardia, Ki67 was not expressed in a comparable manner (Fig. 2B).

**Cervical Neoplasia.** An increasing expression of CFR-1/PAM-1 on cervical epithelium with architectural abnormalities was also observed. The precursors of the cervical squamous cell carcinoma are classified as CIN grade I, II, and III. Mild dysplasias are termed CIN I up to carcinoma in situ lesions CIN III (35). The precursor lesions (CIN I-III) and invasive malignancy of the cervix, the squamous cell carcinoma, were included in this study (Fig. 6).

Similar to earlier observations, normal epithelium showed no reaction with PAM-1 (Fig. 6A), whereas increased staining with PAM-1...
was noted for the different types of cervical neoplasia (Fig. 6, B–D). The staining pattern followed the appearance of atypical cells in the different cell layers and the widening of the basal proliferation zone. The reaction of Ki67 generally correlated with the staining pattern of PAM-1; however, the staining was less intensive.

**Bronchial Carcinogenesis.** Carcinomas of the lung are one of the most frequently occurring carcinomas worldwide. The most common type is the squamous cell carcinoma, which correlates closely with a history of smoking. In the airways of smokers, squamous metaplasia and dysplasia are usually present. In squamous metaplasia the normal bronchial ciliated epithelium is replaced by squamous epithelium. With occurrence of cytological disturbance and severe atypia, the lesion becomes known as squamous dysplasia (36, 37).

Normal ciliated epithelium shows no expression of PAM-1 (Fig. 7A), whereas similar positive PAM-1 reactivities were found in metaplasia and dysplasia of bronchus epithelium. Squamous cell metaplasia of the bronchus represents the initial stages of carcinogenesis and shows a lower intensity of staining compared with dysplasia (Fig. 7B). For dysplasia, the preliminary stage of cancer, a more intensive staining was observed (Fig. 7C). The most intense staining was again observed in the squamous cell carcinoma (Fig. 7D). In each of the three cases in this study, the staining of PAM-1 correlated with the reaction pattern of Ki67.

**Proliferation Zones.** To investigate whether CFR-1/PAM-1 expression is specific for malignant proliferation and not involved in normal proliferative processes (e.g., regeneration of tissue), we performed stainings of different proliferative regions of healthy and premalignant tissue with PAM-1 and Ki67. Fig. 8A shows that the proliferation zone of normal colon mucosa is positive for Ki67 but negative for CFR-1/PAM-1 expression. The same result was seen with normal cervical tissue. Here again Ki67 shows a positive staining of the proliferation zone, whereas CFR-1/PAM-1 is not expressed. In contrast, nondysplastic intestinal-type Barrett’s metaplasia, which defines Barrett’s esophagus, the premalignant lesion for adenocarcinoma of the esophagus (32–34), shows a positive expression of Ki67 and CFR-1/PAM-1. This clearly shows that CFR-1/PAM-1 is not expressed in healthy proliferating cells.

**Summary PAM-1 Expression on Premalignant Lesions.** The immunohistochemical data on precancerous lesions are summarized in Table 2. Three to 10 different cases of each available precursor lesion type were tested. In general, antibody PAM-1 shows a clear positive and homogeneous staining on nearly all of the different precursors, and, in addition, an increasing level of expression with the grade of malignancy. In contrast, the proliferation marker Ki67 shows a similar inhomogeneous expression as on carcinomas (see for example atrophic gastritis, tubular adenoma of stomach, and squamous metaplasia of bronchus), and is expressed on both healthy and malignant tissue. Most importantly, proliferation zones of healthy tissue are clearly positive for Ki67, but negative for PAM-1/CFR-1, proving the association with malignancy.

**DISCUSSION**

Our results demonstrate that CFR-1, which is detected by the fully human antibody PAM-1, is an obviously overexpressed and transcriptionally modified receptor isoform, which is expressed on nearly all of the epithelial cancers of every type and origin. This CFR-1/PAM-1-
specific isoform is not expressed on normal tissue. CFR-1/PAM-1 is also expressed homogeneously on precancerous lesions of the stomach, cervix, colon, esophagus, and lung, and exhibits increasing staining patterns with the escalation of malignancy. CFR-1/PAM-1 is an ideal and reliable diagnostic marker for neoplastic epithelial changes.

Cancers are thought to evolve through a sequence of irreversible genetic alterations, and precancerous lesions are known to be involved in the development of invasive malignancy. Before these alterations, which enable cells to invade the surrounding tissue and to proliferate uncontrolled, abnormalities in the DNA often cause morphological changes that can be recognized as dysplasia. Cell dysplasia is widely regarded as the precursor of numerous carcinomas, such as carcinoma of the esophagus, stomach, colon, cervix, and lung. The best example is the adenoma-carcinoma sequence of colorectal carcinomas. Depending on the size of the adenoma and severity of epithelial dysplasia, tubular adenomas and villous adenomas are at risk of degenerating to carcinomas (38). In addition, the development of ulcerative colitis-related colorectal adenocarcinoma includes the intermediate stages of low- and high-grade dysplasia (30).

Studies have shown that premalignant lesions of the stomach, autoimmune atrophic gastritis, *H. pylori*-associated gastritis, and low- and high-grade dysplasia have increased risks for the development of adenocarcinoma (39). In the United States, 600,000 dysplasias of the cervix are diagnosed every year (400/100,000/year), which is 30 times more than invasive squamous cell carcinomas of the cervix. Approximately 10% of all of the CIN I cases and 20% of CIN II cases progress to CIN III, and at least 12% of CIN III cases progress to invasive carcinomas (35, 40). Squamous metaplasia and dysplasia of the lung are recognized as the preneoplastic stages of squamous cell carcinoma of lung. These stages can be reversible or the situation may become irreversible and proceed to invasive cancer (41).

Therefore, the early detection of precancerous lesions is important for both the therapy and prognosis of the patient. For example, patients with Barrett’s esophagus or Barrett’s dysplasia undergo spe-
cial surveillance endoscopy and biopsy at intervals. Patients whose cancers were discovered during surveillance exhibited an earlier stage of tumor development, and the 5-year survival rate was significantly improved (62%; Ref. 34). There are some assumptions that one-third of all of the patients with high-grade dysplasia in Barrett’s esophagus already have an invasive adenocarcinoma (34). For that reason, early diagnosis is not replaceable for tumor prevention and therapy. Unfortunately, the diagnosis of these preneoplastic stages is difficult, principally because no general guidelines for diagnosis exist. The grading of dysplastic changes is mainly a subjective skill, because grading is based on morphological and cytological changes of cells, e.g., architectural atypias, variation in size and shape of the cells and nuclei, hyperchromatic staining of nuclei, and atypical mitosis to name but a few. It is particularly difficult to distinguish low-grade dysplasia from changes of regenerative mucosa and acute inflammatory damage (1, 30, 34, 40). However, different biomarkers, such as those that detect cell proliferation by immunohistochemical staining, are used for diagnosis and estimation of prognosis. The most commonly used proliferation marker, Ki67, included in this study as a control, does not differentiate between healthy and malignant tissue. Moreover, additional commonly used markers for proliferation, such as PCNA, may be up-regulated in nongrowing cells, which can lead to confusing results (4, 42). PCNA is a highly conserved Mr 36,000 acid nuclear protein, which is responsible for the life and death decision of cells. The sliding clamp protein PCNA is essential for DNA replication, repair, postreplicative processing, and apoptosis. PCNA may be a mechanism to coordinate DNA replication and repair with the cell cycle. PCNA is not only found in various tumors but also in proliferative cells of normal tissue (43–45).

Therefore, there is a clear need for additional markers to support microscopic analysis, especially because most solid cancers are more successfully treated if detected early. The best way to define tumor-specific markers is via human antibodies isolated from cancer patients.
The human monoclonal antibody PAM-1 was isolated from a patient with a stomach carcinoma and defines a new tumor-specific modified version of CFR-1 (14). This CFR-1/PAM-1 receptor is a new tumor-associated proliferation marker, overexpressed and most likely post-transcriptionally modified on nearly all of the investigated carcinomas and precursor lesions, but absent from nonproliferating and proliferating healthy tissue. Compared with the most used proliferation marker Ki67, which exhibits both a heterogeneous and inconsistent expression on premalignant and malignant tissue, CFR-1/PAM-1 is homogeneously and more widely expressed, and in addition, the expression seems to increase with the grade of malignancy. However, the differences on malignant and premalignant tissue between these two markers are not marginal; the more important point is the differential expression of both markers on healthy and malignant proliferating cells.

PAM-1 is a germ-line coded, not affinity maturated IgM antibody that binds to a carbohydrate residue. Most of the tumor-specific monoclonal antibodies thus far detected and described, are germ-line coded IgMs; they are oligoreactive and bind predominantly to carbohydrate structures on tumor cells (10–15). They are most likely produced from CD5/CD110 B cells, and, therefore, belong to the natural (innate) immunity (46–48). Innate immunity has been shown to be responsible for primary recognition and defense of bacteria and viruses, by using specific germ-line coded, and not affinity matured recognition and destruction mechanisms (48–52). The striking parallels of humoral tumor immunity and bacterial immunity might indicate that tumor defense is also the result of innate mechanisms rather than of an affinity maturation process. However, this has to be confirmed by additional investigations, but our data clearly support the observation that conventional human hybridoma technology is an excellent method for the production of diagnostic tools, targets for cancer therapy, and for understanding tumor immunity (53).

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REFERENCES

NEW CFR-1 ISOFORM AS PRECANCEROUS AND CANCEROUS MARKER


Cysteine-rich Fibroblast Growth Factor Receptor 1, a New Marker for Precancerous Epithelial Lesions Defined by the Human Monoclonal Antibody PAM-1

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