[CANCER RESEARCH 53, 3327-3335, July 15, 1993]

Regulation and Heteromeric Structure of the Fibroblast Activation Protein in Normal and Transformed Cells of Mesenchymal and Neuroectodermal Origin[1]

Wolfgang J. Rettig,[2] Pilar Garin-Chesa, John H. Healey, Sai L. Su, Harvey L. Ozer, Manfred Schwab, Anthony P. Albino, and Lloyd J. Old

Immunology Program [W. J. R., S. L. S., A. P. A.], Ludwig Institute for Cancer Research [P. G-C., L. J. O.], Departments of Pathology [P. G. C.] and Surgery [J. H. H.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021; Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, New Jersey 07103 [H. L. O.]; and Deutsches Krebsforschungszentrum, Heidelberg, Germany [M. S.]

ABSTRACT

The human fibroblast activation protein (FAP), defined by monoclonal antibody F19, is expressed *in vivo* in reactive stromal fibroblasts of epithelial cancers, subsets of bone and soft tissue sarcomas, and granulation tissue of healing wounds. FAP is generally absent from the stroma of benign epithelial tumors and normal adult tissues. *In vitro* FAP induction is observed in proliferating cultured fibroblasts and in melanocytes grown with fibroblast growth factor and phorbol ester. In the present study, we show that fibroblast and melanocyte FAP is a cell surface protein comprising noncovalently linked M_r 95,000 (p95) and M_r 105,000 (p105) subunits. In contrast, cultured sarcoma and melanoma cell lines express only p95 or are FAP negative. Immunoblot experiments show that p95, but not p105, carries the epitope defined by monoclonal antibody F19. Furthermore, peptide maps of purified p95 and p105 differ, suggesting that they may be distinct gene products. Loss of FAP or a change from p95/p105 to p95 expression accompanies the acquisition of growth factor independence and tumorigenicity in several *in vitro* test systems, including simian virus 40 transformation of normal fibroblasts, Ha-*ras* transformation of normal melanocytes, supertransformation of osteosarcoma cells, and enhanced N-*MYC* expression in variant neuroblastoma cells, whereas serum-starved normal fibroblasts continue to express p95/p105. Thus, FAP expression appears to be linked to the growth factor-dependent proliferative capacity of normal cells and is not merely a secondary event in proliferating cells; furthermore, FAP expression is inversely correlated with growth factor independence and tumorigenicity in transformed cell lines. This distribution pattern is consistent with a role for p95/p105 in mediating extrinsic, growth regulatory signals in normal cells, possibly as a heteromeric cell surface receptor. Such a physiological function may be obviated when oncogenes with cytoplasmic or nuclear sites of action are activated, reducing extrinsic growth factor dependence and permitting down-regulation of FAP in certain transformed cells.

INTRODUCTION

The invasive growth of malignant tumors and normal wound healing are distinct processes but show a number of histological and biochemical similarities (1–4), including the recruitment of reactive stromal fibroblasts, lymphoid and phagocytic infiltrates, neovascularization, release of peptide mediators and proteolytic enzymes, and induction of an altered ECM.[3] A highly consistent molecular trait shared by tumor stroma and granulation tissue of healing wounds in humans is induction of the FAP, a cell surface molecule of reactive stromal fibroblasts (5, 6) recognized by the mAb F19 (7). Immunohistochemical analysis of normal human tissues has shown that FAP is expressed in some fetal mesenchymal cells but not in adult mesenchyme (5) and that epithelial, lymphoid, and neural cells are FAP^-. This contrasts with other stromal markers, such as tenascin/neuronectin, which show a wider normal tissue distribution (4, 6).

Immunopathological studies have demonstrated FAP expression in a large proportion of bone and soft tissue sarcomas (5). Neoplastic epithelial, neural, and lymphoid cells are FAP^-, but prominent FAP induction is observed in the reactive stromal fibroblasts of many epithelial cancers, including >90% of breast, lung, colorectal, and pancreatic carcinomas (6). Benign and premalignant epithelial tumors, including fibroadenomas and phylloides tumors of the breast and colorectal adenomas, generally lack FAP^+ stromal cells (6). This difference in FAP expression in the stroma of malignant *versus* benign epithelial tumors may be due to the release of FAP-inducing factors from malignant epithelial cells or from tumor-infiltrating lymphocytes or macrophages.

The restricted distribution of FAP in normal tissues and its abundant expression in the stroma of a large proportion of epithelial cancers provide the rationale for developing imaging and immunotherapy strategies aimed at tumor stroma rather than surface molecules of the malignant cells (6). This approach has several potential advantages: (*a*) FAP^+ reactive stromal fibroblasts are very abundant in a large proportion of epithelial cancers, with the stromal compartment making up as much as 90% of the tumor mass in some cases; (*b*) FAP^+ reactive fibroblasts are generally found in close association with tumor capillaries,[4] suggesting that they may be highly accessible to circulating mAbs; (*c*) the same target antigens may be expressed in the stroma of diverse histological types of cancer; and (*d*) outgrowth of antigen-loss variants or mutants is less likely to occur for nontransformed, reactive stromal cells than for malignant tumor cells. As a first step toward defining the targeting potential of mAbs against reactive stromal antigens *in vivo*, Welt *et al.* (8) have shown that ^{131}I-labeled mAb F19 can be used to image hepatic metastases in patients with colorectal carcinomas, due to the induction of FAP^+ stromal fibroblasts in the secondary tumor sites.

Since tumor stroma is a complex, ECM-rich tissue that is not readily dispersed into single-cell suspension, it has been difficult to isolate FAP^+ reactive stromal fibroblasts for direct biochemical and functional analyses. However, short-term cultures of normal fibroblasts, which show *in vitro* FAP induction (5), and several FAP^+ tumor cell lines (5, 7, 9) provide alternative test systems to examine FAP structure, function, and regulation. The present study was designed to characterize the relationship among FAP expression, proliferative activity, and oncogenic transformation in mesenchymal and neuroectodermal cells and to characterize the biochemical nature of FAP.

Received 2/16/93; accepted 5/12/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by the National Cancer Institute (CA-57486, CA-41520, CA-37907), the National Institute on Aging (AG-04821), the Jennie R. and Oliver S. Donaldson Charitable Fund, and the New York City Marathon Fund.

[2] To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

[3] The abbreviations used are: ECM, extracellular matrix; FAP, fibroblast activation protein; FGF, fibroblast growth factor; mAb, monoclonal antibody; IL, interleukin; IL-2R, interleukin-2 receptor; MHA, mixed hemadsorption; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; MEM, minimum essential medium; FBS, fetal bovine serum; NP40, Nonidet P-40; Con A, concanavalin A.

[4] P. Garin-Chesa and W. J. Rettig, unpublished observations.

MATERIALS AND METHODS

Cell Lines and Tissue Culture. Cells were grown in MEM or RPMI 1640 supplemented with 1% nonessential amino acids, 2 mM L-glutamine, antibiotics, and 10–15% FBS. Serum-starved fibroblast cultures were maintained in MEM-0.5% FBS (10). TPA (10 ng/ml; Sigma Chemical Co., St. Louis, MO) and basic FGF (5 ng/ml; R&D Systems, Minneapolis, MN) were used for melanocyte cultures (11). Neuroblastoma variant lines Exp-II and WAC-2 (12) were grown in the presence of G-418 (Geneticin, 400 μg/ml; Sigma). Tumor cell line TE-85, TE-85 subclones (13, 14), and fibroblast strains Hs27 and Hs68 were obtained from the American Type Culture Collection (Rockville, MD). GM series fibroblasts were from the National Institute of General Medical Sciences Cell Repository (Camden, NJ). HAL and AR5, two cloned and immortalized derivatives of fetal diploid bone marrow fibroblasts transformed by SVtsA58, an origin-defective SV40 genome encoding a heat-labile large T-antigen, were previously described (15, 16). Other tumor cell lines and normal fibroblast cultures were from the cell line bank at the Sloan-Kettering Institute or newly established as part of this study.

Monoclonal Antibodies. MAbs F19 (7), AJ2 (17), and TA99 (18) have been described previously. Hybridoma W6/32, producing a mAb against a monomorphic determinant of class I major histocompatibility antigens (HLA-A, -B, -C antigens in humans), was obtained from the American Type Culture Collection. Negative control mAbs were produced in our laboratory.

Serological Procedures. Cells grown in MicroWell plates (Nunc, Roskilde, Denmark) were tested by MHA rosetting assays as described previously (19). For indirect radiobinding assays, cells were grown in Falcon 3047 multiwell plates (1×10^5 cells/well; Becton Dickinson, Lincoln Park, NJ) for 18–24 h, incubated with mAbs for 1 h, washed, and reacted with ^{125}I-labeled goat anti-mouse immunoglobulin (New England Nuclear, Boston, MA) for 30 min. Cells were washed, lysed in SDS-buffer (2% SDS-0.01 M Tris-HCl, pH 7.2), and assayed in a gamma counter.

Immunochemical Procedures. Cells were labeled for 18–24 h with a mixture of [^{35}S]methionine and [^{35}S]cysteine (Tran^{35}S-label, 40 μCi/ml; ICN, Costa Mesa CA) or [^{3}H]glucosamine (40 μg/ml; New England Nuclear) as described before (20) or surface labeled with ^{125}I (500 μCi/25-cm^2 flask; ICN) by the lactoperoxidase method (21). Cell extracts prepared in lysis buffer (0.01 M Tris-HCl-0.15 M NaCl-0.01 M $MgCl_2$-0.5% NP40-20 μg/ml aprotinin-2 mM phenylmethylsulfonyl fluoride, pH 8.0) were used for immunoprecipitation assays, followed by SDS-PAGE and fluorography or autoradiography (19). In some assays, additional protease inhibitors (0.5 mM EDTA, 0.5 μg/ml soybean trypsin inhibitor, 1 μg/ml pepstatin, 3 μg/ml ethyleneglycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 0.12 units/ml α_2-macroglobulin; Sigma) were added to the cell lysis buffer. Immunopurified proteins were analyzed by SDS-PAGE under reducing (sample buffer with 12 mg/ml dithiothreitol; Sigma) or nonreducing conditions (14 mg/ml iodoacetamide; Sigma). For some experiments, extracts of confluent cell cultures were ^{125}I-labeled by the chloramine T method (22, 23), using 100 μCi ^{125}I/100-μl cell lysate; free ^{125}I was removed on a Sephadex-G25 column (Sigma). Lectin chromatography with Con A-Sepharose (Pharmacia) and *Triticum vulgaris* (wheat germ agglutinin)-Sepharose (Sigma) was carried out as described previously (20). In some tests, proteins were digested with neuraminidase (*Vibrio cholerae*; Calbiochem, San Diego CA), endoglycosidase H (25 mIU/ml), *N*-glycanase (10 units/ml), or *O*-glycanase (0.1 units/ml; Genzyme, Cambridge MA) prior to SDS-PAGE analysis (24). For peptide mapping (25), immunopurified ^{125}I-labeled proteins were separated by SDS-PAGE, and specific protein bands were identified by autoradiography, excised, and digested with *Staphylococcus aureus* V8 protease (Sigma). The derived peptides were analyzed by SDS-PAGE and autoradiography.

For immunoblot experiments, NP40 extracts of cultured cells were resolved on SDS-gels, transferred to nitrocellulose (Schleicher & Schuell, Keene NH), and reacted with mAbs; bound mAbs were detected by the alkaline phosphatase procedure (20, 26).

Immunohistochemical Procedures. Tissues were obtained through the Tumor Procurement Service of the Department of Pathology, Memorial Hospital, embedded in OCT compound (Miles, Naperville, IL), snap frozen in isopentane precooled in liquid N_2, and stored at −70°C. Sections (5-μm thick) were cut, mounted on poly-L-lysine-coated slides, air dried, and fixed in

Table 1 *FAP cell surface expression on cultured fibroblasts, sarcomas, and melanocytes determined by MHA rosetting assays with mAb F19*

Target cells		Cell surface reactivity (titer^{-1})	
Derivation	Designation[a]	mAb F19	Control IgG1
Normal tissue-derived fibroblasts			
Fetal lung	GM5387 (p 5–8), GM5389 (p 7–10), F135–35–18 (p 5)	1250[b,c]	–
Fetal skin	GM5386 (p 5–8), GM5388 (p 8–10), F135–60–86 (p 14)	1250	–
Fetal kidney	F135–72–29 (p 6), O-20–69 (p 12)	1250	–
Fetal bone marrow	Hs74	1250	–
Embryonic lung	WI-38 (p 20–25)	1250	–
Newborn foreskin	Hs27 (p 12–16), Hs68 (p 16–26)	1250	–
Adult lung	LU1 (p 4), LU-PA (p 3), LU-RU (p 7)	1250	–
Adult skin	SK-AH (p 2–4), SK-MA (p 2), SK-HO (p 2–3)	1250	–
Adult kidney	KID1 (p 3), KID2 (p 3), KID3 (p 4)	250–1250	–
Adult breast	BR1 (p 1–3), BR2 (p 2–3), BR3 (p 1)	250–1250	–
	BR4 (p 2), BR5 (p 2), BR6 (p 1)	250–1250	–
	BR5 (p 2), BR6 (p 2)	1250	–
Adult bone marrow	BM8 (p 1–3), BM9 (p 1–3)	250	–
Tumor-derived fibroblasts			
Breast cancer	BC1 (p 2), BC2 (p 2), BC3 (p 4), BC4 (p 3)	250–1250	–
Renal cancer	RCC1 (p 3–4), RCC2 (p 4)	250–1250	–
Sarcoma cell lines			
Liposarcoma	SW872	1250	–
Fibrosarcoma	Hs913T	250	–
Rhabdomyosarcoma	Hs729	250	–
Osteosarcoma	TE-85, HOS, A2394	50	–
Fibrosarcoma	HT-1080, 8387	–	–
Rhabdomyosarcoma	RD	–	–
Normal melanocytes			
Fetal foreskin	FSI (p 10)	1250	–
Newborn foreskin	FS234 (p 13–15), FS257 (p 7–10)	250–1250	–
Adult foreskin	FST (p 2)	1250	–

[a] For short-term cultures of normal cells, the passage numbers (p) of the test cultures are listed in parentheses. BR1/BC1, BR2/BC2, BR3/BC3, and BR4/BC4 are paired fibroblast cultures derived from histologically normal breast and breast cancer tissues, respectively, of the same four patients.

[b] Serial 5-fold dilutions of F19 hybridoma tissue culture supernatant (starting dilution, 1:2) or unrelated negative control mouse IgG1 (starting concentration, 5 μg/ml) were tested by MHA assay on viable target cells. Numbers in the body of the table refer to the highest mAb dilutions giving positive reactions (*i.e.*, rosette formation) with the target cells; –, no reactivity at the highest concentration of mAb tested.

[c] For all mAb F19 reactive cell types listed, antigen expression was detected on >95% of cells within the test cultures as determined microscopically by the fraction of target cells showing rosette formation with MHA indicator cells (19).

acetone (4°C, 10 min). MAbs were used at 10–20 μg/ml, and the avidin-biotin immunoperoxidase procedure was performed as described before (4, 6, 20).

RESULTS

FAP Expression in Cultured Normal Fibroblasts

Short-term cultures of normal human fibroblasts derived from adult (skin, breast, kidney, bone marrow), newborn (foreskin), fetal (skin, lung, kidney, bone marrow), or embryonic tissues (lung) or from the stroma of epithelial cancers (breast, kidney) express cell surface FAP as determined by MHA (Table 1) and radiobinding assays (Fig. 1). No discernible differences in FAP levels distinguished fibroblasts derived from FAP^- (adult skin, breast, kidney) and FAP^+ tissues (breast cancer stroma) or fibroblasts of fetal and adult origin. Additional radiobinding assays showed that Hs27 and Hs68 normal foreskin fibroblasts, grown to confluence and maintained in low-serum media for up to 10 days to achieve growth arrest, continued to express significant levels of FAP (>75% of binding in serum-supplemented cultures). Similarly, immunoprecipitation tests with metabolically labeled, growth-arrested normal Hs27 fibroblasts showed continued FAP synthesis (see below). Unlike cultured normal fibroblasts, epithelial cancer cell lines are FAP^-, and melanoma cell lines show variable FAP expression (Fig. 1).

FAP Expression in Transformed Mesenchymal Cells

SV40-transformed Fibroblasts. The effect of SV40 transformation and immortalization on FAP expression was studied in several cell lines (Fig. 2). We found that the SV40-transformed and immortalized W18-VA2 fibroblasts express far lower levels of FAP than the parental normal WI-38 fibroblasts. Similarly, HAL and AR5, two immortalized lines derived from normal bone marrow fibroblasts transformed with SVtsA58, an origin-defective SV40 genome encoding a heat-labile large T-antigen, express lower levels of FAP than the nontransformed bone marrow fibroblasts. When temperature-sensitive, transformed HAL and AR5 cells were grown for 24–72 h at the nonpermissive temperature for large T-antigen function, FAP expression remained unchanged at the same low level.

Sarcoma Cell Lines. We used MHA (Table 1) and radiobinding assays to identify FAP^+ and FAP^- sarcoma cell lines, including the FAP high-expressor line SW872, osteosarcomas TE-85 and HOS, which express moderate levels of the antigen, and HT-1080, which is

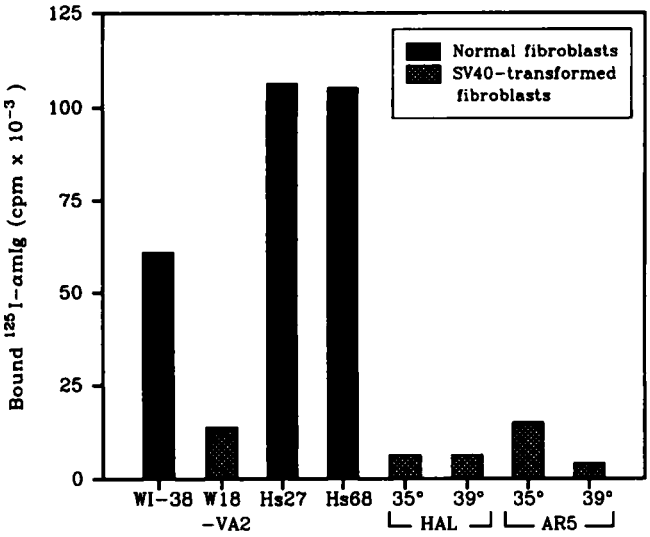

Fig. 2. FAP cell surface expression on cultured normal fibroblasts and SV40-transformed, immortalized fibroblast cell lines determined by radiobinding assays with mAb F19. Assays were carried out as described in Fig. 1, except that parallel cultures of HAL and AR5 were grown either at the permissive temperature (35°C) for large T-antigen function or at the nonpermissive temperature (72 h at 39°C) prior to binding assays.

FAP^-. Further tests were carried out with subclones of HOS that differ in their growth characteristics (13, 14). The parental HOS cells show low saturation density *in vitro* and are not tumorigenic in nude mice. In contrast, the subclone KHOS/NP, derived from HOS by transformation with the v-Ki-*ras* oncogene, and subclone MNNG/HOS, derived from HOS by mutagenesis with MNNG, grow to high densities *in vitro* and are highly tumorigenic in mice; two nontumorigenic revertants of KHOS/NP, designated KHOS/240S and KHOS/312H, have also been derived. Radiobinding assays showed that the tumorigenic lines KHOS/NP and MNNG/HOS are FAP^-, whereas the nontumorigenic revertants KHOS/240S and KHOS/312H reexpress FAP at levels similar to the parental HOS cells.

Biochemical Characterization of FAP in Normal and Transformed Mesenchymal Cells

Fibroblasts. FAP expression in the Hs27 and GM5387 normal fibroblasts was examined by immunoprecipitation tests with NP40 extracts of cultures metabolically labeled with Trans^{35}S-label or [^{3}H]-

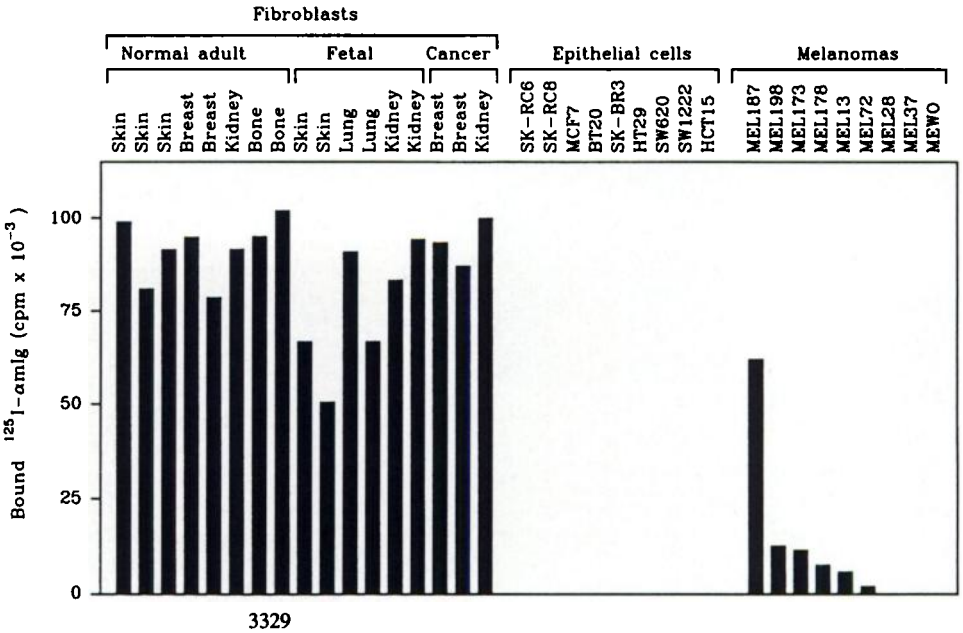

Fig. 1. Cell surface expression of FAP on cultured fibroblasts, carcinoma, and melanoma cell lines determined by radiobinding assays with mAb F19. Fibroblasts were obtained from normal adult skin (strains SK-AH, SK-MA, SK-HO), adult breast (BR1, BR2), adult kidney (KID1), adult bone marrow (BM8, BM9), fetal skin (GM5386, GM5388), fetal lung (F135–35–18, GM5387), fetal kidney (F135–72–29, O-20–69), breast cancers (BC1, BC2), or kidney cancer (RCC1). Cells plated in Falcon 3047 multiwell plates (1×10^5 cells/well) and cultured for 24 h at 37°C in a humidified chamber (95% air/5% CO_2) were tested with mAb F19 or negative control mouse IgG1, followed by ^{125}I-rabbit anti-mouse immunoglobulin (^{125}I-αmIg). After extensive washing, bound radioactivity was removed with SDS buffer (2% SDS-Tris-HCl 10 mM, pH 7.2) and assayed in a gamma counter. Specific binding data [cpm bound in F19 wells minus cpm bound in negative control wells (Bound ^{125}I-αmIg)] are shown for representative experiments; nonspecific binding was $<2 \times 10^3$ in all assays.

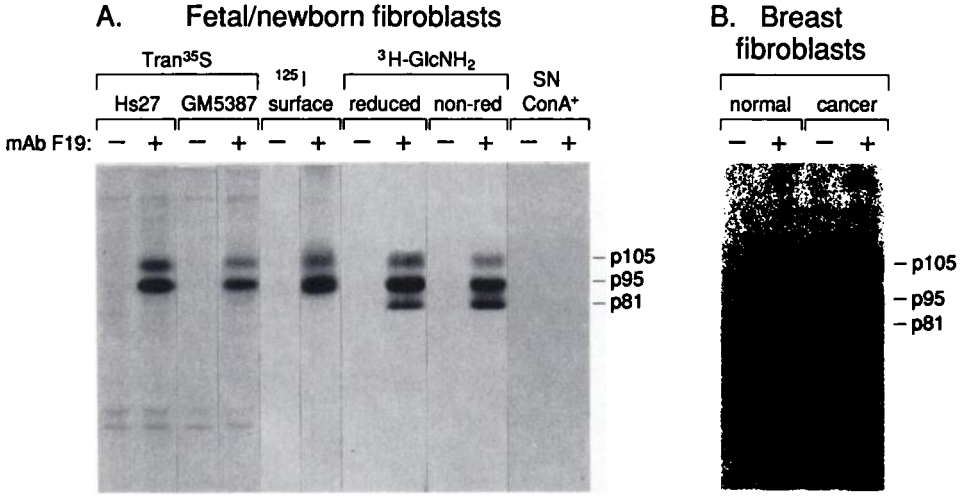

Fig. 3. Immunoprecipitation analysis of FAP in cultured fibroblasts derived from normal and cancer tissues. Cell extracts or cell-free culture supernatants (SN) were tested with mAb F19 (+) or negative control mouse IgG1 (−). *A, lanes 1* and *2*, tests with Hs27 newborn foreskin fibroblasts, which were grown in MEM-0.5%FBS for 7 days prior to metabolic labeling with Tran^{35}S-label; *lanes 3–12*, tests with GM5387 fetal lung fibroblasts, either metabolically labeled with Tran^{35}S-label (Tran^{35}S), surface iodinated by the lactoperoxidase method (^{125}I-surface), or metabolically labeled with [^{3}H]glucosamine (^{3}H-GlcNH$_2$); in addition, the Con A-bound fraction of Tran^{35}S-labeled GM5387 cultures was tested. *B*, comparison of FAP proteins immunoprecipitated from cultured fibroblasts derived from paired samples of normal and cancerous tissues of the same patient. Immunoprecipitates were separated on SDS-gels under reducing conditions, except for one experiment run under nonreducing (non-red) conditions. *Far right lane*, size of FAP proteins as determined with molecular size markers.

glucosamine (Fig. 3*A*). These assays revealed two major protein species of M_r 95,000 (p95) and M_r 105,000 (p105), respectively, with an additional band of M_r 81,000 (p81) seen in some experiments; the same protein species were also obtained from ^{125}I-labeled NP40 extracts. The p95 and p105 protein species, but not p81, were observed when intact fibroblasts were surface labeled with ^{125}I prior to extraction, indicating that only p95 and p105 are exposed on the cell surface. SDS-gels run under reducing and nonreducing conditions produced identical protein patterns, with no evidence for intermolecular or intramolecular disulfide bonds. FAP was not detected in the spent media of metabolically labeled fibroblast cultures, even when highly concentrated, Con A-bound fractions were tested. Immunoprecipitation tests with additional normal fibroblast strains (GM series, WI-38, Hs68, F-SK-AH; Table 1) showed uniform p95/p105 expression in these cells. Tests with paired fibroblast cultures derived from normal breast tissues (FAP$^-$ *in vivo*) and cancerous breast tissues (FAP$^+$ *in vivo*) of three individuals showed comparable levels of p95 and p105 expression (Table 1, Fig. 3*B*), consistent with *in vitro* FAP induction in the normal breast-derived fibroblast cultures.

SV40-transformed Fibroblasts. The SVtsA58-transformed fibroblast clones HAL and AR5 grow as immortalized cell lines at 35°C, the permissive temperature for large T-antigen function, but stop proliferating at 39°C, the nonpermissive temperature. Radiobinding assays showed low levels of cell surface FAP expression on these cells at either temperature (Fig. 2). Metabolic labeling and immunoprecipitation tests confirmed these findings, showing low levels of p95 and lack of p105 expression at the permissive and nonpermissive temperatures (Fig. 4).

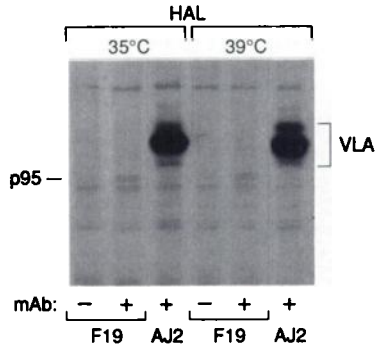

Fig. 4. Immunoprecipitation analysis of FAP and VLAβ1-integrin expression in SVtsA58-transformed HAL cells grown at either the permissive (35°C) or nonpermissive temperature (39°C) for large T-antigen function (39°C), respectively. Cells were grown at the indicated temperatures for 48 h prior to metabolic labeling with Tran^{35}S-label for 24 h. Extracts were tested with (+) or without mAb F19 (−) or with mAb AJ2 (anti-VLAβ1), and immunoprecipitates were separated on SDS-gels under reducing conditions. The positions of FAPα (p95) and the VLAβ1 proteins are indicated.

Sarcomas. Extracts of metabolically labeled sarcoma cell lines SW872, HOS, A2394, and Hs913T, which are FAP$^+$ in MHA and radiobinding assays, were used for immunoprecipitation tests with mAb F19. The p95 subunit of FAP was detected in each of these cell lines, with no evidence of p105, as illustrated in Fig. 5 for SW872 cells. No FAP was found in SW872 culture supernatants. When intact SW872 cells were surface labeled with ^{125}I prior to extraction, only p95 was detected; however, when SW872 cells were first solubilized with detergent and the extracted proteins labeled with ^{125}I, both p95 and p81 were identified. This pattern suggests that only p95 is exposed on the cell surface. Since p81 carries the epitope defined by mAb F19 (see below) and is labeled with [^{3}H]glucosamine, it may be a partially glycosylated precursor of p95.

Lectin chromatography showed that FAP derived from fibroblasts or SW872 sarcoma cells binds to Con A (Fig. 5*A*) and wheat germ agglutinin. Digestion of p95 derived from SW872 with endoglycosidase H, an enzyme that catalyzes the hydrolysis of the chitobiose core of high mannose and certain hybrid oligosaccharides, yielded an M_r 92,000 protein species, and neuraminidase digestion produced an M_r 89,000 species. *N*-Glycanase, which cleaves all N-linked oligosaccharide side chains, produced an M_r 75,000 species, p75 (Fig. 5*B*). *O*-Glycanase, which cleaves all O-linked oligosaccharides, had no effect on the electrophoretic mobility of p95 on SDS-gels. These results suggest that p95 comprises a p75 polypeptide, with sialylated, N-linked carbohydrate side chains but no evidence of *O*-glycans.

p95 and p105 Subunits of FAP Are Antigenically and Biochemically Distinct

Immunoblot analysis of cultured fibroblasts and SW872 was used to determine which FAP subunits are recognized by mAb F19. As shown in Fig. 6, mAb F19 reacts with p95 and p81 in fibroblast and sarcoma cell extracts but not with the p105 subunit present in fibroblast extracts. To define the relationship between p95 and p105, we purified p95 and p105 from ^{125}I-labeled fibroblast extracts by immunoprecipitation with mAb F19 and excision of specific bands from SDS-gels. Isolated p95 and p105 proteins were digested with *Staphylococcus aureus* V8 protease, and the resulting peptides were separated on a 15% SDS-gel and detected by autoradiography. These experiments showed that p95 and p105 have unique peptide maps (Fig. 7). Taken together with the fact that only p95 is recognized by mAb F19 in immunoblot experiments, these observations suggest that

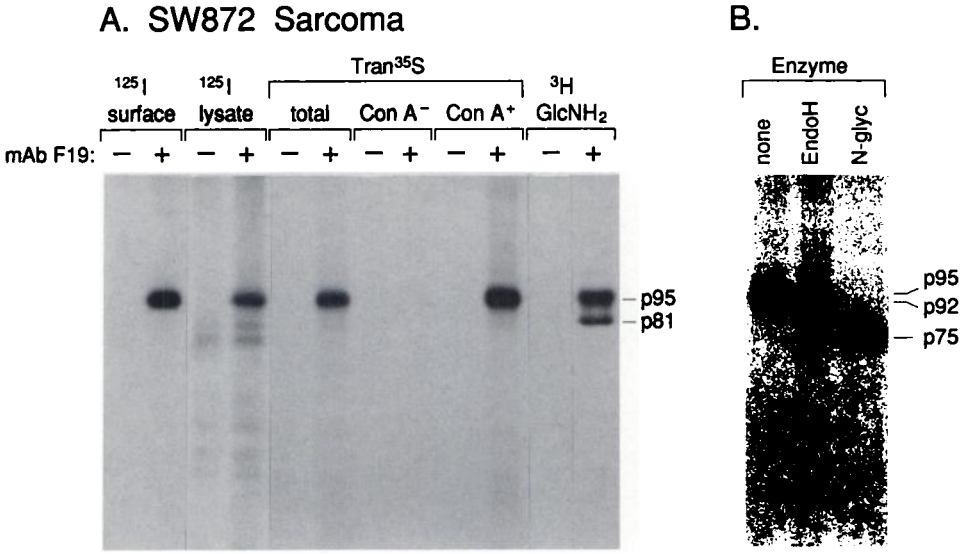

Fig. 5. Immunoprecipitation analysis of FAP in SW872 liposarcoma cells. Cell extracts were tested with mAb F19 (+) or negative control mouse IgG1 (−). *A*, SW872 cells labeled by one of the following methods: surface labeling of intact cells by the lactoperoxidase method (^{125}I surface), ^{125}I labeling of NP40-solubilized cell extracts by the chloramine T method (^{125}I-lysate), or metabolic labeling with Tran^{35}S-label (Tran^{35}S) or [^{3}H]glucosamine (^{3}H $GlcNH_2$). For Tran^{35}S-labeled extracts, unfractionated (total), Con A-bound (Con A$^+$), and Con A-unbound (Con A$^-$) fractions were tested. *B*, FAP proteins immunopurified from Tran^{35}S-labeled cells digested with endoglycosidase H (EndoH) or *N*-glycanase (N-glc) prior to SDS-gel electrophoresis.

p95 and p105 are distinct proteins, possibly encoded by separate genes, which associate on the surface of certain cell types through noncovalent, non-disulfide bonds.

FAP Expression in Cultured Melanocytic and Neural Cells

Melanocytic Cells. Four independently derived cultures of normal skin melanocytes were tested by MHA assays for FAP cell surface expression (Table 1). FAP was detected on fetal (FSI), newborn (FS234, FS257), and adult (FST) melanocytes. Immunoprecipitation tests with metabolically labeled FS234 and FS257 cultures revealed the presence of p95 and p105 (Fig. 8). To determine the effect of oncogenic transformation on FAP expression in melanocytic cells, we examined normal melanocytes infected with a retrovirus containing the viral Ha-*ras* oncogene (11). The derived cell line, 10W*ras*/early, shows reduced growth factor dependence, forms colonies in soft agar, but is nontumorigenic in mice. These cells express reduced levels of FAP in MHA assays and, in immunoprecipitation tests, show p95 but no p105 expression (Fig. 8). The 10W*ras*/early cells, after serial passaging in culture for several months, have given rise to a fully transformed melanocyte line, 10W*ras*/late. 10W*ras*/later cells are characterized by aneuploidy, specific chromosome abnormalities, growth factor-independent growth *in vitro*, and tumorigenic potential in nude mice. These cells show no detectable FAP in either MHA or immunoprecipitation assays (Fig. 8). This stepwise loss of p105 and p95 during experimental melanocyte transformation *in vitro* is antigen specific since 10W*ras*/early and 10W*ras*/late cells continue to express melanocyte antigens such as VLAβ1 and HLA class I and have been shown previously to express elevated levels of other proteins and glycolipid antigens (11).

MHA, radiobinding, and immunoprecipitation assays were used to examine FAP expression in nine melanoma cell lines. One melanoma line was strongly FAP$^+$, five lines showed moderate or low levels of expression, and three lines were FAP$^-$. In immunoprecipitation tests with the FAP$^+$ lines, only p95 was detected (Fig. 8*B*).

Variant Neuroblastoma Cells. Typical neuroblastoma cells with neuroblastic features lack FAP expression. However, some neuroblastoma cell lines transdifferentiate into glial/melanocytic or ectomesenchymal, S-type cells *in vitro* (9, 27). These S-type cells commonly show reduced proliferative potential and low tumorigenicity and may express FAP (9), as illustrated in Fig. 9 for SH-EP, a cloned derivative of the FAP$^-$ neuroblastoma cell line SK-N-SH. SH-EP expresses low levels of the endogenous N-*MYC* protooncogene, but Schweigerer *et al.* (12) have generated SH-EP subclones with enhanced expression of an exogenous N-*MYC* gene (12), WAC2 and EXPII, which are less dependent on exogenous growth factors and show increased tumorigenicity in *nu/nu* mice. Immunoprecipitation tests showed that WAC2 (Fig. 9) and EXPII cells lose FAP expression but continue to express

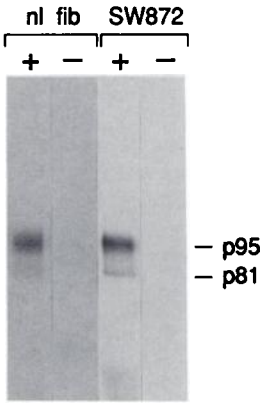

Fig. 6. Immunoblot analysis of FAP proteins in extracts of cultured normal fibroblasts (nl fib) and SW872 sarcoma cells. Cell extracts were separated on SDS-gels, transferred to nitrocellulose sheets, and tested with mAb F19 (+) or unrelated negative control mouse IgG1 (−) using the alkaline phosphatase detection assay.

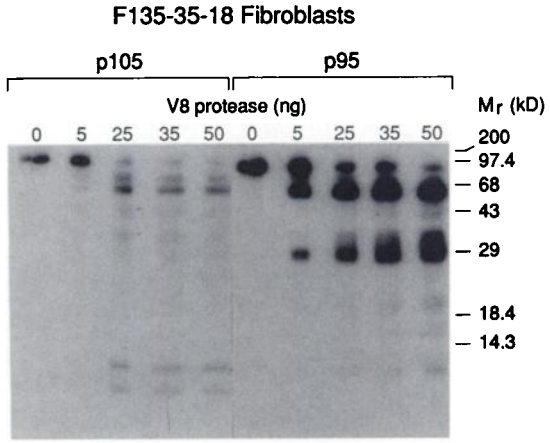

Fig. 7. One-dimensional peptide maps of ^{125}I-labeled, immunopurified p105 (*left*) and p95 subunits (*right*) of FAP. Aliquots of p105 and p95 were digested with different amounts of *S. aureus* V8 protease as indicated, separated on a 15% SDS-gel, and detected by autoradiography. *Far right lane*, positions of molecular size markers run on the same gel.

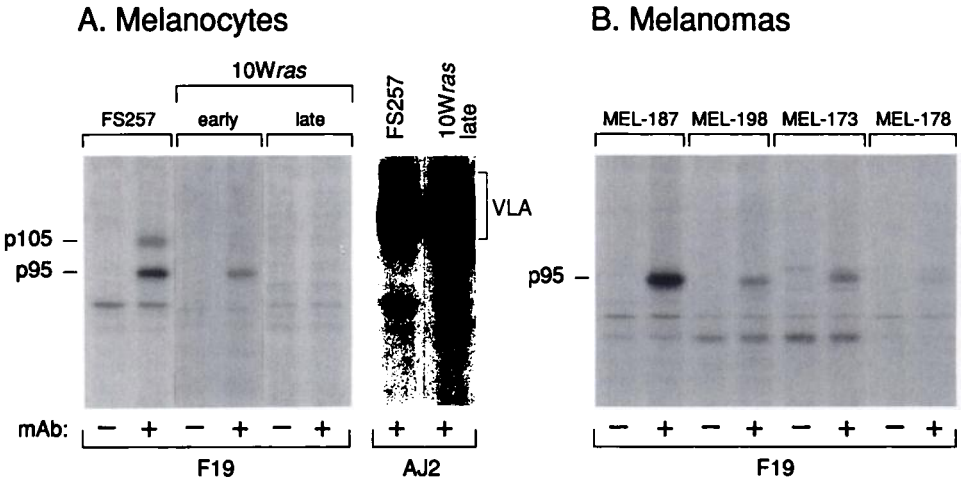

Fig. 8. Immunoprecipitation analysis of FAP in normal and transformed melanocytic cells. *A*, short-term culture of normal melanocyte strain FS257 (passage 8), early-passage Ha-*ras*-transformed melanocytes (10W*ras*/early, passage 33), and late-passage, fully transformed melanocytes (10W*ras*/late, passage 134) tested with mAb F19 (+), negative control mouse IgG1 (−), or mAb AJ2 (anti-VLAβ1). *B*, immunoprecipitation tests with four melanoma cell lines showing FAP expression in radiobinding assays.

several other cell surface proteins of S-type cells (9), such as HLA class I and VLAβ1-integrins.

FAP Expression *in Vivo*

Immunohistochemical methods were used to detect FAP expression in paired samples of normal and neoplastic breast tissues obtained from five patients, in normal skin, and in melanocytic tumors. We confirmed that normal breast tissues are FAP$^-$ and that breast cancer stroma shows abundant FAP$^+$ reactive fibroblasts (Fig. 10*A–D*); included in this analysis were two of the paired samples of normal and neoplastic breast tissues used to establish the fibroblast cultures described above. Tests with a large number of fetal (8 weeks gestational age to full term) and adult skin samples obtained from multiple body sites failed to detect FAP in either normal melanocytes or dermal fibroblasts, whereas positive control experiments with mAb TA99 (18) showed the expected melanocytes labeling. (A detailed account of these studies will be presented elsewhere.) We examined three nevi for FAP expression, including one intradermal nevus and two compound nevi, and the intradermal nevus showed homogeneous FAP immunoreactivity (Fig. 10*E*). Work is in progress to determine the frequency of FAP expression in a larger series of benign and premalignant melanocytic lesions. Of 20 malignant melanomas tested, none showed FAP immunoreactivity (Fig. 10*F*).

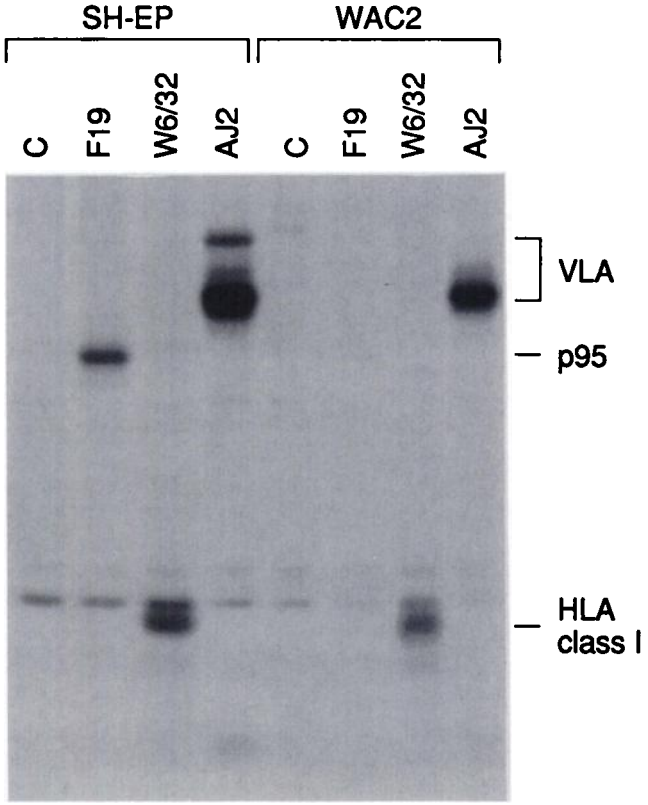

Fig. 9. Immunoprecipitation analysis of FAP, HLA class I, and VLAβ1-integrin expression in S-type neuroblastoma cell line SH-EP and in the SH-EP subclone, WAC2, expressing elevated levels of N-*MYC* protooncogene. Tests were carried out with negative control mouse IgG1 (C), mAb F19, mAb W6/32 (anti-HLA class I), and mAb AJ2 (anti-VLAβ1).

DISCUSSION

FAP induction in reactive fibroblasts of healing wounds and cancer stroma *in vivo* (5, 6) suggests that the molecule may be part of a proliferative program of fibroblastic cells, and FAP induction in proliferating, cultured fibroblasts derived from FAP$^-$ normal tissues is consistent with this idea. However, the present study clearly shows that FAP expression is not merely a secondary phenomenon in proliferating fibroblasts. For example, growth arrest of FAP$^+$ normal fibroblasts in low-serum media does not lead to loss of FAP expression, and rapid growth of SV40-transformed fibroblasts is accompanied by decreased rather than increased FAP levels. Furthermore, normal fibroblasts transformed and immortalized with SVtsA58, an origin-defective SV40 genome encoding a temperature-sensitive large T-antigen (15, 16), show low levels of FAP at both the permissive temperature for large T-antigen function, when the cells divide rapidly, and the nonpermissive temperature, when the cells are no longer capacitated for growth in serum-supplemented media and stop proliferating.

The analysis of established sarcoma cell lines confirmed that FAP expression in transformed mesenchymal cells is not uniformly high, as might be expected for a secondarily growth-regulated molecule (10, 28); instead, antigen levels range from high (*e.g.*, liposarcoma SW872) or intermediate (*e.g.*, osteosarcoma TE-85) to undetectable (*e.g.*, fibrosarcoma HT-1080). Furthermore, TE-85 subclones supertransformed with v-Ki-*ras* or MNNG (13, 14) and revertants of v-Ki-*ras*-transformed TE-85 cells revealed reciprocal changes for FAP expression and *in vitro* growth or tumorigenicity in athymic *nu/nu* mice. Thus, parental TE-85 cells are FAP$^+$, grow to low saturation density *in vitro*, and are nontumorigenic. In contrast, TE-85 cells transformed with v-Ki-*ras* or MNNG are FAP$^-$, grow to high density *in vitro*, and are highly tumorigenic; FAP is reexpressed in slow-growing, nontumorigenic revertants of the v-Ki-*ras*-transformed TE-85 subclones.

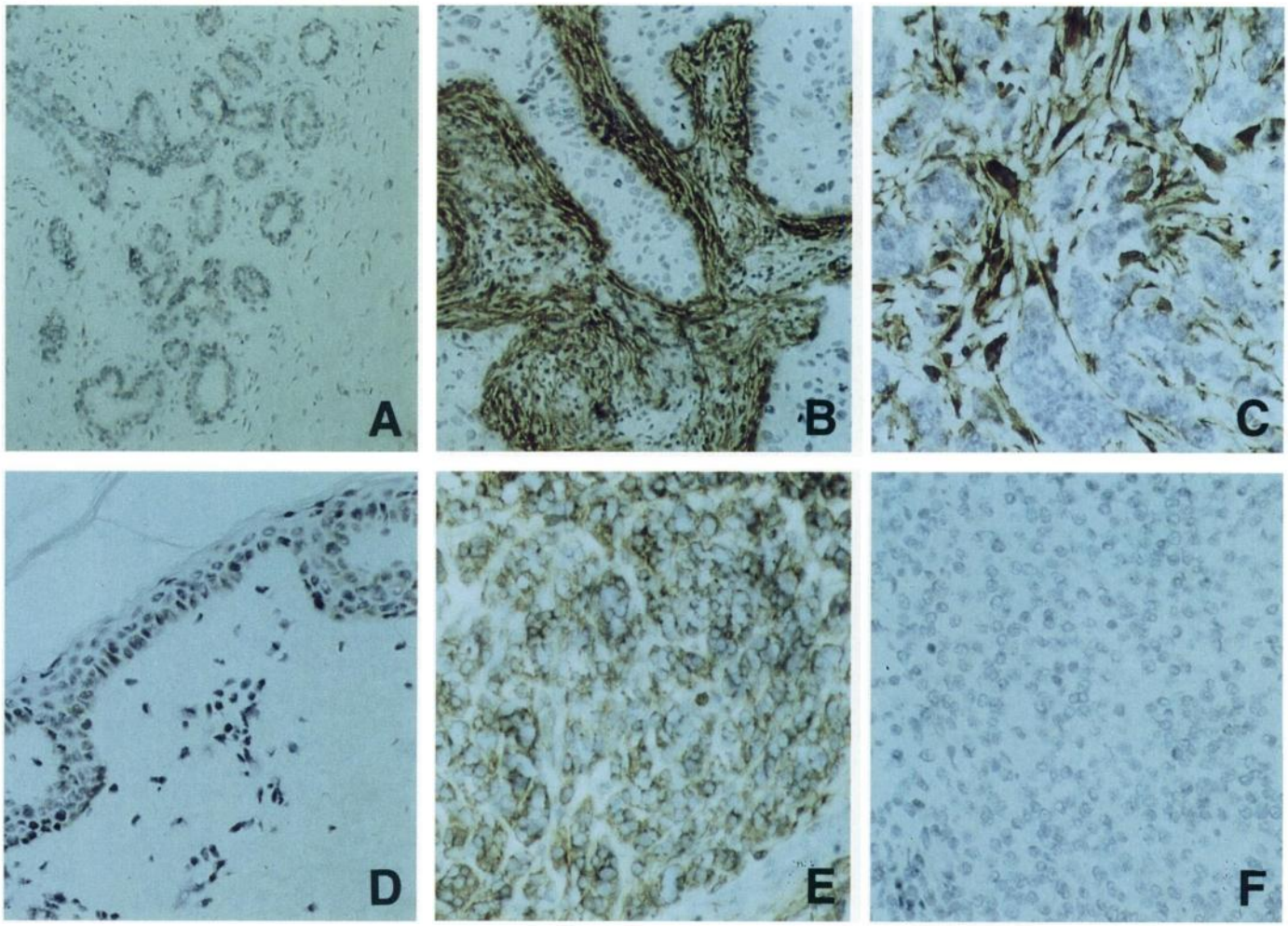

Fig. 10. Immunohistochemical detection of FAP in normal and neoplastic tissues. *A*, normal breast tissue tested with mAb F19; no immunostaining. *B*, primary breast cancer tested with mAb F19; immunostaining of reactive stromal fibroblasts but not malignant epithelial cells. *C* and *D*, invasive breast cancer infiltrating the dermal layer of breast skin (*C*) and adjacent area of normal skin (*D*) showing prominent induction of FAP immunoreactivity in the reactive stromal fibroblasts of the malignant lesion. *E*, intradermal nevus with uniform FAP immunostaining of the nevus cells. Avidin-biotin immunoperoxidase staining with hematoxylin counterstaining. Negative control experiments with unrelated mAbs were included in all assays (not shown) and did not produce immunostaining. Original magnifications: *A*, *B*, and *D*, × 100; *C*, *E*, and *F*, × 200.

Confirming this reciprocal pattern, the fibrosarcoma line HT-1080 is FAP$^-$, grows to high density *in vitro*, and is highly tumorigenic (29), whereas the liposarcoma line SW872 is FAP$^+$, attains low density *in vitro*, and is poorly tumorigenic.

The present study identifies two biochemically distinct forms of FAP expressed in disparate cell types. Normal fibroblast FAP comprises two subunits, p95 (referred to as α-subunit, FAPα) and p105 (β-subunit, FAPβ), which are linked by noncovalent, non-disulfide bonds. We show that FAPα is the subunit recognized by mAb F19. FAPβ is not recognized by this mAb but, instead, is detected by virtue of its association with FAPα. The α-subunit was found to comprise a M_r 75,000 polypeptide and a significant amount of sialylated, N-linked oligosaccharides, with no evidence of *O*-glycans. FAPβ is glycosylated, as shown by [^{3}H]glucosamine labeling, but its carbohydrate content has not yet been determined. Both FAPα and FAPβ can be labeled with ^{125}I on intact cells, indicating that they are exposed on the cell surface. In contrast, the p81 species, which is seen in immunoprecipitation and immunoblot experiments with mAb F19, is not labeled on intact cells, suggesting that it is a partially glycosylated, intracellular precursor of FAPα. Since FAPα and FAPβ are antigenically distinct and show different peptide maps, they are probably distinct proteins, possibly encoded by separate genes. Unlike cultured normal fibroblasts, SV40-transformed fibroblasts and FAP$^+$ tumor cell lines express only FAPα, without detectable FAPβ.

We have previously reported that a subset of cultured melanoma and neural tumor cell lines and cultured leptomeningeal cells, all of which originate from the neural crest, express FAP but that the corresponding tissues are FAP$^-$ *in vivo* (5–7, 9). The present study extends these observations by showing that normal skin melanocytes cultured in the presence of FGF and TPA express FAP and by identifying FAP$^+$ melanocytic cells in an intradermal nevus. Thus, FAP expression in melanoma cell lines may not be a simple tissue culture artifact or related to their transformed phenotype. Instead, FAP may be part of a physiological, adaptive program of normal melanocytes that is induced by tissue culture factors. A similar adaptive response may be induced in some, but not all, melanomas grown in tissue culture. Whether FAP is expressed *in vivo* during specific stages of normal neural crest development, *e.g.*, in fetal melanoblasts or melanocyte precursors migrating from the neural crest to the skin, remains to be determined, and identification of FAP homologues in species with well-studied neural crest pathways (30, 31) may open up this line of investigation.

Similar to our findings for normal and transformed mesenchymal cells, we show that cultured normal melanocytes express FAPα/β, whereas malignant melanoma cell lines are FAP$^-$ or express only the α-subunit. Furthermore, in a two-step, *in vitro* model of melanoma pathogenesis in which v-Ha-*ras* transformation of normal cultured melanocytes leads to partial growth factor independence (32), and

subsequent chromosomal abnormalities lead to a fully transformed phenotype (11, 32), we observed successive stages of FAP loss. The early-passage, v-Ha-*ras*-transformed melanocytes, which grow as long-term cell lines but remain TPA dependent and are nontumorigenic in *nu/nu* mice, retain expression of FAPα but lose the associated β-subunit. Upon long-term culture, these cells acquire chromosomal abnormalities typical for malignant melanoma, become TPA independent and tumorigenic, and lose FAPα expression. Loss of FAP is not the only surface antigenic change observed during melanocyte transformation. For example, previous studies with human and murine melanocytes have demonstrated the loss of adenosine deaminase-binding protein and reduced expression of the c-*kit* product following transformation (11, 32–34). Conversely, transformed melanocytes show enhanced expression of antigens such as G_{D3} ganglioside, MHC class II, and epidermal growth factor receptors (11, 32).

The physiological role of FAP in melanocytes or reactive fibroblasts is still unknown, but it can be compared to other known fibroblast and melanocyte antigens. For example, a large number of cell surface receptors for soluble ligands, ECM proteins, and cell adhesion molecules are expressed in cultured fibroblasts and melanocytes, including several integrin-type ECM receptors (35), and receptors for platelet-derived growth factor (36), transferrin (28), epidermal growth factor (37), fibroblast growth factors (38), hepatocyte growth factor (39). Several of these receptors show a multisubunit structure, but they clearly differ from FAP biochemically and in tissue specificity.

Findings for the high-affinity IL-2R of T-lymphocytes (40) may provide a precedent for certain properties of FAP. Like FAP, IL-2R was first described as an activation antigen, namely, the T-cell activation antigen Tac. Resting T-cells are IL-2R$^-$ but become IL-2R$^+$ after specific antigen binding or T-cell antigen receptor cross-linking; IL-2R on these cells comprises distinct α- and β-subunits encoded by separate genes. Activated IL-2R$^+$ T-cells fail to proliferate unless stimulated by a second signal, which is provided by exogenous IL-2. While the IL-2/IL-2R system is essential for normal T-cell proliferation, only a subset of T-cell leukemias and lymphomas is IL-2 dependent, and neoplastic T-cells may express high levels of IL-2Rα/β, or lack IL-2R, or express individual receptor subunits (40, 41). It is tempting to speculate that the conversion of resting fibrocytes to activated, proliferating fibroblasts also occurs in discrete steps, each triggered by distinct signals. The first signal may cause FAP$^-$ fibrocytes to enter a program of activation that includes *de novo* synthesis of FAPα/β but does not by itself cause proliferation. While these FAP$^+$ fibroblasts may be capacitated for cell growth, they will only divide in response to a second signal, which may be provided by known mitogenic peptides or by as yet undefined factors. Conceivably, FAP itself may act as a receptor for such a putative second signal. This model could explain why fibroblasts retain their FAPα^+/β^+ phenotype in the absence of cell proliferation, since growth arrest in serum-depleted media or at confluence is reversible and does not alter the growth-capacitated state of the cells. Conversely, FAP expression may be down-regulated if its normal function is obviated by activation of cytoplasmic or nuclear oncogene products that render the malignant cells independent of extrinsic, growth regulatory signals.

ACKNOWLEDGMENTS

We thank W. J. Lovenvirth for technical assistance and Dr. G. Gastl for providing kidney fibroblasts.

REFERENCES

1. Dvorak, H. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med., *315:* 1650–1659, 1986.
2. Sporn M. B., and Roberts, A. B. Peptide growth factors and inflammation, tissue repair, and cancer. J. Clin. Invest., *78:* 329–332, 1986.
3. Folkman, J. Tumor angiogenesis. Adv. Cancer Res., *43:* 175–203, 1985.
4. Garin-Chesa, P., Melamed, M. R., and Rettig, W. J. Immunohistochemical analysis of human neuronectin expression in normal, reactive, and neoplastic tissues. J. Histochem. Cytochem., *37:* 1767–1776, 1989.
5. Rettig, W. J., Garin-Chesa, P., Beresford, H. R., Oettgen, H. F., Melamed, M. R., and Old, L. J. Cell surface glycoproteins of human sarcomas: differential expression in normal and malignant tissues and cultured cells. Proc. Natl. Acad. Sci. USA, *85:* 3110–3114, 1988.
6. Garin-Chesa, P., Old, L. J., and Rettig, W. J. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody-target in human epithelial cancers. Proc. Natl. Acad. Sci. USA, *87:* 7235–7239, 1990.
7. Rettig, W. J., Garin-Chesa P., Beresford, H. R., Feickert, H. J., Jennings, M. T., Cohen, J., Oettgen, H. F., and Old, L. J. Differential expression of cell surface antigens and glial fibrillary acidic protein in human astrocytoma subsets. Cancer Res., *46:* 6406–6412, 1986.
8. Welt, S., Rettig, W., Divgi, C., Garin-Chesa, P., Cohen, A., Finstad, C., Larson, S., Finn, R., and Old, L. J. Phase I study of ^{131}I-monoclonal antibody F19 detecting an activation antigen of neoplastic stroma. Proc. Am. Assoc. Cancer Res., *33:* 319, 1992.
9. Rettig, W. J., Spengler, B. A., Garin-Chesa, P., Old, L. J., and Biedler, J. L. Coordinate changes in neuronal phenotype and surface antigen expression in human neuroblastoma cell variants. Cancer Res., *47:* 1383–1389, 1987.
10. Klein, C. E., Ozer, H. L., Traganos, F., Atzpodien, J., Oettgen, H. F., and Old, L. J. A transformation-associated 130-kD cell surface glycoprotein is growth controlled in normal human cells. J. Exp. Med., *167:* 1684–1696.
11. Albino, A. P., Sozzi, G., Nanus, D. M., Jhanwar, S. C., and Houghton, A. N. Malignant transformation of human melanocytes: induction of a complete melanoma phenotype and genotype. Oncogene, *7:* 2315–2321, 1992.
12. Schweigerer, L., Breit, S., Wenzel, A., Tsunamoto, K., Ludwig, R., and Schwab, M. Augmented MYCN expression advances the malignant phenotype of human neuroblastoma cells: evidence for induction of autocrine growth factor activity. Cancer Res., *50:* 4411–4416, 1990.
13. Cho, H. Y., and Rhim, J. S. Cycloheximide-dependent reversion of human cells transformed by MSV and chemical carcinogen. Science (Washington DC), *205:* 691–693, 1979.
14. Rhim, J. S., Park, D. K., Arnstein, P., Huebner, R. J., Weisburger, E. K., and Nelson-Rees, W. A. Transformation of human cells in culture by N-methyl-N'-nitro-N-nitrosoguanidine. Nature (Lond.), *256:* 751–753, 1975.
15. Radna, R. L., Caton, Y., Jha, K. K., Kaplan, P., Li, G., Traganos, F., and Ozer, H. L. Growth of immortal simian virus 40 *tsA*-transformed human fibroblasts is temperature dependent. Mol. Cell. Biol., *9:* 3093–3096, 1989.
16. Hubbard-Smith, K., Patsalis, P., Pardinas, J. R., Jha, K. K., Henderson, A. S., and Ozer, H. L. Altered chromosome 6 in immortal human fibroblasts. Mol. Cell. Biol., *12:* 2273–2281, 1992.
17. Rettig, W. J., Dracopoli, N. C., Goetzger, T. A., Spengler, B. A., Biedler, J. L., Oettgen, H. F., and Old, L. J. Somatic cell genetic analysis of human cell surface antigens: chromosomal assignments and regulation of expression in rodent-human hybrid cells. Proc. Natl. Acad. Sci. USA, *81:* 6437–6441, 1984.
18. Thomson, T. M., Mattes, J. M., Roux, L., Old, L. J., and Lloyd, K. O. Pigmentation-associated glycoprotein of human melanomas and melanocytes: definition with a mouse monoclonal antibody. J. Invest. Dermatol. *85:* 169–174, 1985.
19. Rettig, W. J., Nishimura, H., Yenamandra, A. K., Seki, T., Obata, F., Beresford, H. R., Old, L. J., and Silver, J. Differential expression of the human Thy-1 gene in rodent-human somatic cell hybrids. J. Immunol., *138:* 4484–4489, 1987.
20. Rettig, W. J., Hoffman, S., Su, S. L., and Garin-Chesa, P. Species diversity of neuronectin and cytotactin expression patterns in the vertebrate central nervous system. Brain Res., *590:* 219–228, 1992.
21. Harlow, E., and Lane, D. Antibodies: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1988.
22. Sambrock, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning, Ed. 2. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
23. Kantor, R. R. S., Mattes, M. J., Lloyd, K. O., Old, L. J., and Albino, A. P. Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. J. Biol. Chem., *262:* 15158–15165, 1987.
24. Rettig, W. J., Garin-Chesa, P., Healey, J. H., Su, S. L., Jaffe, E. A., and Old, L. J. Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. Proc. Natl. Acad. Sci. USA, *89:* 10832–10836, 1992.
25. Cleveland, D. W., Fischer, S. G., Kirscher, M. W., and Laemmli, U. K. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem., *252:* 1102–1106, 1977.
26. Fellinger, E. J., Garin-Chesa, P., Su, S. L., De Angelis, P., Lane, J. M., and Rettig, W. J. Biochemical and genetic characterization of the HBA71 Ewing's sarcoma cell surface antigen. Cancer Res., *51:* 336–340, 1991.
27. Ross, R. A., Spengler, B. A., and Biedler, J. L. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. J. Natl. Cancer Inst., *71:* 741–747, 1983.
28. Trowbridge, I. S., and Omary, M. B. Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. Proc. Natl. Acad. Sci. USA, *78:* 3039–3043, 1981.
29. Rasheed, S., Nelson-Rees, W. A., Toth, E. M., Arnstein, P., and Gardner, M. Characterization of a newly derived human sarcoma cell line (HT-1080). Cancer (Phila.), *33:* 1027–1033, 1974.
30. Weston, J. A. Neural crest cell development. *In:* Embryonic Development, Part B: Cellular Aspects, pp. 359–379. New York: Alan R. Liss, 1982.
31. Anderson, D. J. The neural crest cell lineage problem: neuropoiesis? Neuron, *3:* 1–12, 1989.
32. Houghton, A. N., Albino, A. P., Cordon-Cardo, C., Davis, L. J., and Eisinger, M. Cell

surface antigens of human melanocytes and melanomas. Expression of adenosine deaminase binding protein is extinguished with melanocyte transformation. J. Exp. Med., *167:* 197–212, 1988.
33. Lassam, N., and Bickford, S. Loss of c-kit expression in cultured melanoma cells. Oncogene, *7:* 51–56, 1992.
34. Funasaka, Y., Boulton, T., Cobb, M., Yarden, Y., Fan, B., Lyman, S. D., Williams, D. E., Anderson, D. M., Zakut, R., Mishima, Y., and Halaban, R. c-Kit-kinase induces a cascade of protein tyrosine phosphorylation in normal human melanocytes in response to mast cell growth factor and stimulates mitogen-activated protein kinase but is down-regulated in melanomas. Mol. Biol. Cell, *3:* 197–209, 1992.
35. Hynes, R. O. Integrins: versatility, modulation, and signaling in cell adhesion. Cell, *69:* 11–25, 1992.
36. Matsui, T., Heidaran, M., Mik, T., Popescu, N., La Rochelle, W., Kraus, M., Pierce, J., and Aaronson, S. Isolation of a novel receptor DNA establishes the existence of two PDGF receptor genes. Science (Washington DC), *243:* 800–804, 1989.
37. Real, F. X., Rettig, W. J., Garin-Chesa, P., Melamed, M. R., Old, L. J., and Mendelsohn, J. M. Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation. Cancer Res., *46:* 4726–4731, 1986.
38. Becker, D., Lee, P. L., Rodeck, U., and Herlyn, M. Inhibition of the fibroblast growth factor receptor 1 (FGFR-1) gene in human melanocytes and malignant melanomas leads to inhibition of proliferation and signs indicative of differentiation. Oncogene, *7:* 2303–2313, 1992.
39. Halaban, R., Rubin, J. S., Funusaka, Y., Cobb, M., Boulton, T., Faletto, D., Rosen, E., Chan, A., Yoko, K., White, W., Cook, C., and Mollmann, G. Met and hepatocyte growth factor/scatter factor signal transduction in normal melanocytes and melanoma cells. Oncogene, *7:* 2195–2206, 1992.
40. Waldman, T. A. The interleukin-2 receptor. J. Biol. Chem., *266:* 2681–2684, 1991.
41. Hoshino, S., Oshimi, K., Tsudo, M., Miyasaka, M., Teramura, M., Masuda, M., Motoji, T., and Mizoguchi, H. Flow cytometric analysis of expression of interleukin-2 receptor β chain (p70–75) on various leukemic cells. Blood, *76:* 767–774, 1990.