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Orofacial and gastrointestinal hyperplasia and neoplasia in *smad4*$^{+/-}$ and *elf*$^{+/-}$/*smad4*$^{+/-}$ mutant mice

Robert S. Redman[1], Varalakshmi Katuri[2], Yi Tang[2], Allan Dillner[1], Bibhuti Mishra[2], Lopa Mishra[1,2]

[1]*Department of Veterans Affairs Medical Center, Washington, DC;* [2]*Laboratory of GI Developmental Biology, Department of Surgical Sciences and Medicine, Lombardi Cancer Center, Georgetown University, Washington, DC, USA*

BACKGROUND: Smad4 is vital to the roles of Smads 2 and 3 in transforming growth factor-beta (TGF)-β signal transduction, and inactivated Smad4 is common to human gastrointestinal cancers. The embryonic liver fodrin (ELF) is a β-spectrin that facilitates the nuclear translocation of activated Smad4.

METHODS: *Smad4*$^{+/-}$ mice, known to develop gastrointestinal cancer, were crossbred with *elf*$^{+/-}$ mice. The *smad4*$^{+/-}$ and *smad4*$^{+/-}$/*elf*$^{+/-}$ offspring were autopsied as abnormalities developed.

RESULTS: In addition to polyps and adenocarcinomas of the stomach and duodenum, the *smad4*$^{+/-}$ mice developed squamous cell carcinomas of the skin, oral mucosa and forestomach, benign neoplasms of connective tissue and lacrimal gland, and a lymphoma. The *smad4*$^{+/-}$/*elf*$^{+/-}$ mice developed extensive hyperplasia and neoplasia of the gastric mucosa.

CONCLUSION: These findings indicate that investigating interactions among *smad4*, *elf*, and other genes involved in TGF-β signaling should be useful in further delineating the processes of neoplasia in a wide variety of tissues.

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Keywords: ELF β-spectrin; hyperplasia; mouse, mutant strain; neoplasms; Smad4 protein; transforming growth factor-β

Introduction

The transforming growth factor-beta (TGF)-β family of cytokines regulates many essential cellular functions, including differentiation, proliferation, migration, and apoptosis (1). Another family of proteins, the Smads, plays crucial roles in TGF-β signal transduction and propagation (1–3). The receptor-activated Smads 1–3 are phosphorylated in response to ligand-induced activation of type I receptors, which then form heteromic complexes with Smad4, the common Smad. The complexes transfer into the nucleus, where, by interacting with DNA-binding proteins, they regulate the transcription of specific genes. The central part Smad4 plays in ensuring appropriate cellular responses to TGF-β signaling has been demonstrated by the striking effects of both inadequate and excessive expression. Inactivation of Smad4 by homozygous deletion or other mutations is a common feature of human carcinomas of the pancreas, colon and lung, and is generally associated with poorer differentiation histologically and poorer prognosis clinically (1, 4–6). Colorectal cancers expressing Smad4 respond better to chemotherapy with 5-fluorouracil than do those without Smad4 expression (7). On the contrary, some hepatocellular cancers over-express Smad4 (8). In experiments with *smad4*-deficient mice, those with homozygous loss of *smad4* did not survive early embryogenesis (9), and many of those with heterozygous loss developed gastric and duodenal polyps, with some of the former progressing to adenocarcinoma (10). Other workers found that the adenomatous polyps that develop in mice heterozygous for the *Apc* gene progressed to malignancy in mice heterozygous for both the *Apc* and *smad4* genes (11). However, in contrast to the previously cited work, no gastrointestinal or other abnormalities developed in the mice heterozygous for *smad4* alone.

Membrane-associated cytoskeleton components such as spectrin have been shown to be important in the maintenance of a number of aspects of cell differentiation in the adult, such as cell shape and organelle movement (12). These observations suggested that spectrins should have an important role in differentiation during development. This was borne out by experiments which demonstrated a vital role for the ELF isoform of embryonic liver fodrin (ELF), a novel β-spectrin, in embryonic liver and brain development (13–15). Subsequent production of mice deficient in ELF showed that ELF is essential to overall development, as homozygous loss of *elf* resulted in mid-gestational death (16). Mice with heterozygous loss of *elf* were phenotypically normal up to age 9 months. Subsequently, however, some of these mice have developed neoplasms at age 1 year (unpublished data). An important additional finding of this study was that without ELF, the nuclear translocation of Smad3 and Smad4 was disrupted.

Correspondence: Robert S. Redman, Oral Pathology Research Laboratory (151-I), Department of Veterans Affairs Medical Center, 50 Irving Street, NW, Washington, DC 20422, USA. Tel.: 202-745-8490. Fax: 202-462-2006. E-mail: oralpath@erols.com
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Our initial objective was to further monitor and analyze the development of hyperplasia, neoplasia and other abnormalities in *smad4*$^{+/-}$ mice. We present here the unexpected occurrence of dysplasia and neoplasia of tissues other than those in the lower gastrointestinal tract, including squamous cell carcinoma and lymphoma of the head and neck, in almost half the smad4$^{+/-}$ mice. We also had begun to explore the role of ELF in the gastrointestinal tumorigenesis seen in *smad4*$^{+/-}$ mice. To that end, we crossed *smad4*$^{+/-}$ with *elf*$^{+/-}$ mice, and compared our findings in the hybrid with those of the *smad4*$^{+/-}$ mice. The preliminary results were so consistent and instructive that they also are presented at this time.

Materials and methods

Experimental animals

All animal procedures were approved by the Internal Animal Care and Use Committee of the Washington, DC, Department of Veterans Affairs Medical Center. Animals were housed in 'shoe box' cages with bedding in a room with controlled temperature and humidty, and had unlimited access to a commercial pelleted diet (Teklad, Rodent Diet 8604, Harlan Teklad, Madison, WI, USA) and tap water. Pups were weaned at age 21 days. Mice carrying targeted disruption of *smad4*, kindly provided by Dr Chuxia Deng (NIDDK, NIH), were interbred with wild-type mice of the original stock (Black Swiss; Taconic, Taconic Quality Laboratory Animals, Germantown, NY, USA). The resulting mutant heterozygotes, previously referred to as *Smad4*$^{ex8/+}$ and *Smad4*$^{8+/-}$ because the disruption occurred in exon 8 of *Smad4* (9, 10), are designated *smad4*$^{+/-}$ in the present paper. Identification of *smad4*$^{+/-}$ mice was by Southern analysis, as previously described (9).

The production and identifications of mice with targeted disruption of *elf* (*elf*$^{+/-}$) were as previously described (16). Both methods were employed to identify hybrid mutant (*smad4*$^{+/-}$/*elf*$^{+/-}$) mice.

Procedures

Smad4$^{+/-}$ mice were autopsied when visible or palpable tumors or other abnormalities developed. However, when tumors occurred at unexpectedly early ages (5–9 months) in several, the next four *smad4*$^{+/-}$ and the first six *smad4*$^{+/-}$/*elf*$^{+/-}$ mice to reach the same age range were autopsied without waiting for overt abnormalities to develop. At autopsy, the esophagus, exorbital lacrimal glands, heart, kidney, liver, pancreas, stomach, small intestine (portions of duodenum, ileum and jejunum), major (parotid, sublingual and submandibular) salivary glands, and any grossly abnormal tissues were sampled for histologic analysis. Normal tissues representative of the age range, sexes and tissue samples of the autopsied mutant mice were obtained for reference from seven mice (two *elf*$^{+/-}$ and five wild type).

Tissues were cleansed of blood, food and feces, fixed for 14 h in 4.0% formaldehyde (prepared fresh from paraformaldehyde) in 0.1 M NaH_2PO_4, pH 7.4, with 2.5% dimethylsulfoxide and 0.001 M $CaCl_2$, and processed and embedded in paraffin (melting point 56°C). Stomachs either were bisected and flattened during fixation or were expanded with fixative by clamping the adjacent duodenum and esophagus and infusing the lumen via a syringe. Representative 6 μm sections were stained with hematoxylin and eosin (H & E) for histologic examination.

Results

The samples consisted of 16 (eight male and eight female) *smad4*$^{+/-}$ mice, ages 5–23 months, and six (four male and two female) *smad4*$^{+/-}$/*elf*$^{+/-}$ mice, ages 7–9 months. A considerable range of abnormalities was encountered, often with more than one in a given mouse, and these are summarized in Table 1. Representative photomicrographs of benign abnormalities are shown in Fig. 1, and of malignancies, in Fig. 2.

In mouse, the stomach has two main chambers, divided morphologically by a circular internal ridge and functionally by a sphincter. The anterior chamber is lined by aglandular, keratinized stratified squamous epithelium, similar to that of the esophagus. The transition to the glandular epithelium of the posterior chamber occurs abruptly just past the ridge (17). In the present paper, the anterior and posterior chambers will be referred to as the forestomach and glandular stomach, respectively, to avoid confusion with the terminology of the human stomach, all of which has glandular mucosa (18).

Benign abnormalities

Patchy hyperplasia and polyps of the duodenal mucosa, including one instance of hyperplastic Brunners glands (Fig. 1a, b), and of the glandular stomach (Fig. 1c) occurred in *smad4*$^{+/-}$ mice beginning at 8 months. The gastric, but not duodenal, hyperplasia became both more extensive and thicker, and increasingly punctuated by polyps, with increasing age. In all six *smad4*$^{+/-}$/*elf*$^{+/-}$ mice, the gastric glandular mucosa grossly was much thicker, and histologically more extensively hyperplastic (Fig. 1d), than in the *smad4*$^{+/-}$ mice in the same age range. Hyperkeratosis but not hyperplasia of the forestomach epithelium occurred in a few mice of both mutant types, usually when there was also an extensive hyperplasia of the glandular stomach, or in one instance, adjacent to a neoplasm (Fig. 2c). Abscesses and other abnormalities of skin were rather common, including a region of sebaceous hyperplasia, cysts, and inflammation in a *smad4*$^{+/-}$ mouse (Fig. 1e). Two *smad4*$^{+/-}$ mice had a large abscess of the testis with reactive regional lymph nodes, and another had an abscessed bladder. One *smad4*$^{+/-}$ mouse had a mass on the foreleg, which zwas composed of an outer layer of collagenous tissue, a myxomatous middle layer, and an inner layer of adipose tissue. A paucity of inflammatory cells and its appearance after puberty indicated that this was neither a reactive lesion nor a hamartoma, but rather, a neoplasm (fibromyxolipoma).

Abnormalities also were seen in several exocrine organs. Hyperplasia and dysplasia of a Harderian

Table 1 Neoplasias, hyperplasias and other abnormalities in *Smad4*$^{+/-}$ and *Smad4*$^{+/-}$/*elf*$^{+/-}$ mutant mice

Number	*Sex*	*Age*	*Weight (g)*	*Abnormalities*
				Smad4$^{+/-}$
3176	F	4	17	SCCA of right mandible and maxilla, origin in oral mucosa; hyperkeratosis of forestomach and esophagus; abscess and reactive lymph node in skin of left neck
3160	F	5	16	SCCA of right mandible, origin in overlying skin
3045	M	8	16	SCCA of right mandible, origin in oral mucosa; hyperkeratosis and SCCA of forestomach; focal hyperplasia of glandular stomach
3082	M	9	44	Fibromyxolipoma of leg
3092	M	9	36	None
3095	M	9	38	None
3118	M	9	42	Large polyp of duoedenum; hyperplastic Brunner's glands
3135	M	9	36	None
3103	F	9	37	Abscess of bladder
3171	M	9	28	Abscesses of external ear and testis; exophthalmos of left eye associated with hyperplasia and dysplasia of Harderian lacrimal gland; focal dilation and squamous metaplasia (non-keratinizing) of left Stenson's (parotid) duct
3035	M	11	36	Focal hyperplasia of glandular mucosa of stomach; abscess of testis with reactive lymph node
3017	M	13	42	Focal hyperplasia and polyp of glandular mucosa of stomach
3084	F	17	33	SCCA of external ear and scalp
3042	F	22	39	Extensive hyperplastic mucosa and polyps of duodenum and glandular stomach
3021	F	22	38	Hyperplastic mucosa and polyps of duodenum and glandular stomach; large foci of periductal lymphocytes in parotid and submandibular salivary glands and peri-islet lymphocytes in pancreas
3014	F	23	34	Hyperplastic mucosa and polyps of duodenum and glandular stomach; adenocarcinoma of stomach; lymphoma, large cell, with plasmacytoid cells, of cervical lymph node; large foci of periductal lymphocytes of submandibular salivary glands
				Smad4$^{+/-}$/*elf*$^{+/-}$
3108	M	7	38	Extensive hyperplasia and a small adenocarcinoma of glandular mucosa of stomach; hyperplasia of submandibular glands
3128	M	8	42	Sebaceous hyperplasia and cysts of skin overlying mandible; hyperplasia of glandular mucosa of stomach; duodenal polyp; hyperplasia of submandibular glands
3058	F	9	30	Hyperplasia of glandular mucosa of stomach; marked hyperkeratosis of forestomach; foci of periductal lymphocytes of submandibular glands
3100	F	9	43	Hyperplasia and microcysts of glandular mucosa of stomach
3106	M	9	36	Hyperplasia of glandular mucosa of stomach; hyperplasia of submandibular glands; foci of peri-islet lymphocytes in pancreas
3114	M	9	34	Hyperplasia of glandular mucosa of stomach; marked hyperkeratosis of forestomach; hyperplasia of submandibular glands; foci of periductal lymphocytes in exorbital lacrimal gland

Hyperplasia, generalized or focal thickening or enlargement due to increased number of normal cells; polyp, tubular adenoma; SCCA, squamous cell carcinoma.

lacrimal gland (Fig. 1e) caused marked exophthalmus in a *smad4*$^{+/-}$ mouse. A dilated segment of Stenson's (parotid) duct with non-keratinized stratified squamous epithlium occurred on the ipsilateral side of the same mouse (Fig. 1f). This was not due to pressure on the duct from the enlarged Harderian gland, as other parts of the duct were not dilated and the acini of the gland were not atrophic. There were focal accumulations of lymphocytes around the large ducts of the salivary and lacrimal glands in several of the *smad4*$^{+/-}$ and *smad4*$^{+/-}$/*elf*$^{+/-}$ mice, and around the islets of Langerhans in a pancreas in one *smad4*$^{+/-}$/*elf*$^{+/-}$ mouse. The submandibular glands of all four male *smad4*$^{+/-}$/*elf*$^{+/-}$ mice were grossly much larger, but histologically indistinguishable from, those of the male *smad4*$^{+/-}$ and normal mice of the same age. No dysplastic or adenomatous areas were seen in these glands.

Malignancies

Three *smad4*$^{+/-}$ mice developed squamous cell carcinoma, which invaded half or more of the mandible, and, in one, also invaded the maxilla. Grossly, the involved part of the mandible was greatly enlarged, and histologically, the bone was almost completely replaced by the cancerous epithelium and supporting stroma (Fig. 2a). Two of these had continuity with oral epithelium but not skin (Fig. 2b), and one had continuity with skin but not oral epithelium. A squamous cell carcinoma also developed in the forestomach of one of the mice with mandibular cancer (Fig. 2c, d). A fourth mouse developed squamous cell carcinoma of the skin of the ear and scalp which was exophytic and did not invade the underlying cartilage or bone. Within each of these squamous cell carcinomas there were poorly, moderately and well-differentiated areas (e.g. Fig. 2c, d).

Gastric adenocarcinomas developed in one *smad4*$^{+/-}$ (Fig. 2e) and one *smad4*$^{+/-}$/*elf*$^{+/-}$ mouse. The *smad4*$^{+/-}$ mouse also had a large cell lymphoma in a greatly enlarged cervical lymph node (Fig. 2f). In addition, a number of polyps in the gastric and duodenal mucosa in the *smad4*$^{+/-}$ mice had foci of dysplasia.

Discussion

Smad4$^{+/-}$ *mice*

A major contribution of the present work is that it extends the range of tissues affected by hyperplasia and neoplasia in *smad4*$^{+/-}$ mice from gastric and intestinal

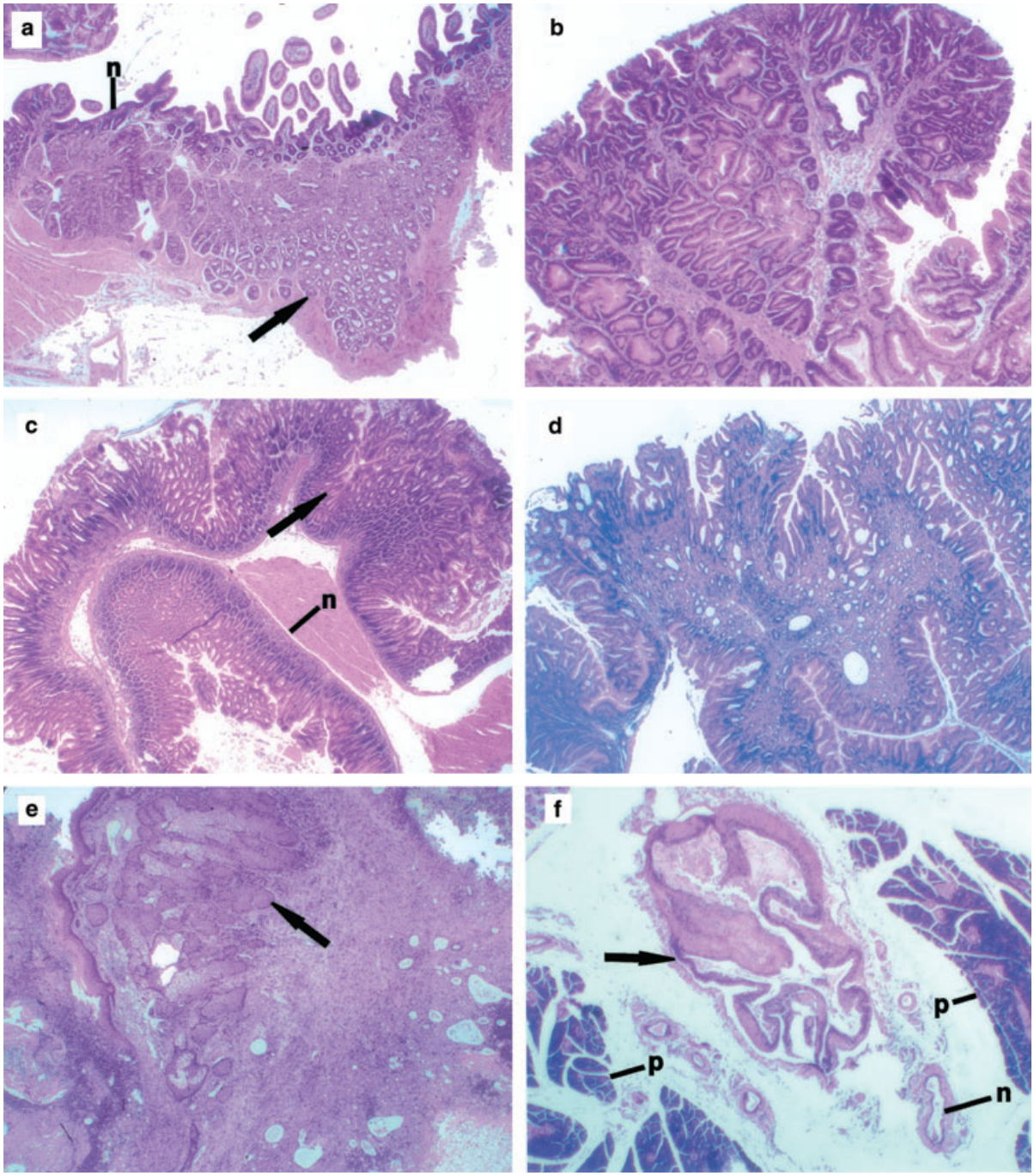

Figure 1 Photomicrographs showing hyperplasia of gastrointestinal mucosa and skin in $smad4^{+/-}$ and $elf^{+/-}/smad4^{+/-}$ mutant mice. (a) Focal hyperplasia (arrow) of Brunners glands (n = normal) and (b) a large polyp in the duodenum of a male $smad4^{+/-}$ mouse at age 9 months. (c) Focal hyperplasia (arrow) of the gastric glandular mucosa (n = normal) in a male $smad4^{+/-}$ mouse at age 13 months. (d) Polypoid hyperplasia involves almost all of the gastric glandular mucosa in a male $elf^{+/-}/smad4^{+/-}$ mouse at age 7 months. (e) Hyperplasia (arrowheads), inflammation and microcysts of sebaceous glands of the perioral skin in a male $elf^{+/-}/smad4^{+/-}$ mouse at age 8 months. (f) Dilation and squamous metaplasia (arrow) of a segment of Stensen's duct in a male $smad4^{+/-}$ mouse at age 9 months. A normal segment of Stensen's duct (n) and associated parotid gland (p) are nearby. Hematoxylin and eosin (H & E). Magnification: a, c, d and e = ×27; b and f = ×40.

epithelia to oral mucosal, salivary and skin epithelia, and lymphatic and connective tissues. Further, squamous cell carcinoma has not been observed previously in $smad4^{+/-}$ mice. These additions greatly expand the potential utility and value of these mice as a model for studying the relationships among TGF-β signaling, *smad4* deficiency, and neoplasia. In this regard, it is noteworthy that decreased expression of TGF-β-related Smad proteins has been implicated in basal and squamous cell carcinoma of human skin (19). In addition, decreased Smad4 expression has been common in, and associated with more advanced stages of, squamous cell

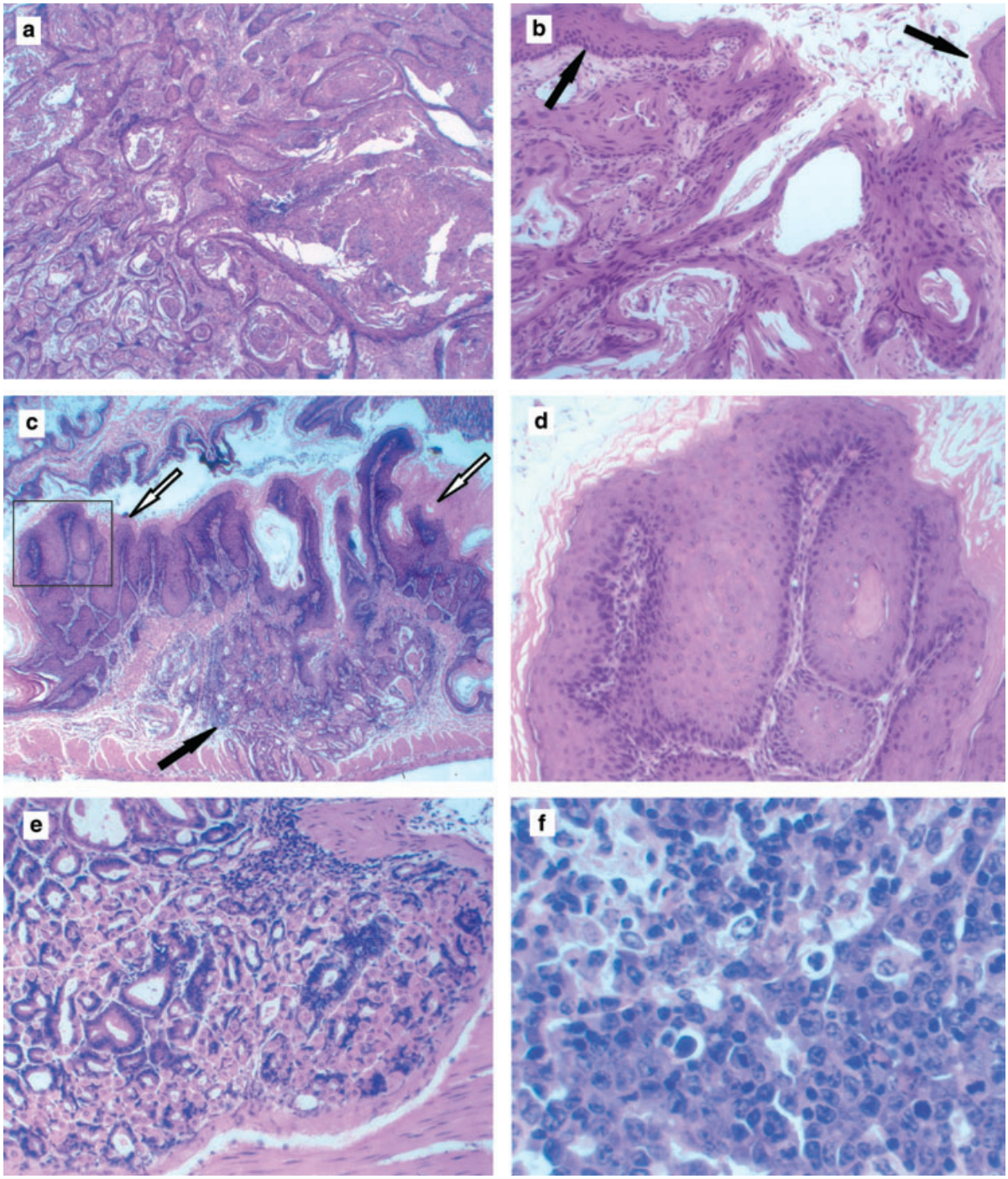

Figure 2 Photomicrographs of malignant neoplasms in *smad4*$^{+/-}$ and *elf*$^{+/-}$/*smad4*$^{+/-}$ mutant mice. (a) The mandible of a female *smad4*$^{+/-}$ mouse at age 8 months is riddled with squamous cell carcinoma, which (b) has continuity with dysplastic, hyperplastic and hyperkeratotic oral mucosal epithelium (arrows). (c) In the forestomach of the same mouse is a squamous cell carcinoma with well-differentiated (open arrows) and moderately to poorly differentiated (solid arrow) areas. The area bounded by the rectangle is shown at higher magnification in (d). (e) A small adenocarcinoma is in the gastric glandular mucosa of a male *elf*$^{+/-}$/*smad4*$^{+/-}$ mouse at age 7 months. (f) A large cell lymphoma with plasmacytoid features is in a cervical lymph node in a female *smad4*$^{+/-}$ mouse at age 23 months. Hematoxylin and eosin (H & E). Magnification: a and c = ×27; b and e = ×110; d = ×150; f = ×430.

carcinoma of the esophagus (20, 21). However, in small samples, a very low frequency of Smad mutations has been found in most squamous cell carcinomas of the head and neck (22), including the esophagus (23). This suggests that alterations in pathways other than Smad gene mutations may result in decreased Smad4 expression in human squamous cell carcinomas of the head and neck. Furthermore, disruption of TGF-β signaling

can occur in numerous other ways. Reduced expression of TGF-β1 and/or one of its receptors, TβR-l was demonstrated in all but a tiny minority of more than 100 human oral/pharyngeal/laryngeal squamous cell carcinomas (24). In two human oral cancer-derived keratinocyte cell lines which are resistant to TGF-β1-induced G_1 arrest, Smad4 was undetectable, yet Smads 2 and 3 were fully expressed (25). Treatment with TGF-β1 resulted in phosphorylation of Smad2, but attenuation of cell growth occurred only with high doses of TGF-β1 or restoration of Smad4 by transfection.

Except for the squamous cell carcinoma of the forestomach, the observed hyperplasia and neoplasia of the gastrointestinal tract in the *smad4*$^{+/-}$ mice generally are similar in histologic type and chronology of development to those reported by Xu et al. (10). This was anticipated, as some of their mice were obtained to establish our breeder colony of *smad4*-deficient mice. Xu and colleagues (10) noted that 'TGF-β1 and its receptors have been most strongly implicated in gastric cancer formation', and that 'SMAD4 is a common mediator for all TGF-β family members'. They also noted that 'because there are no amplification steps between membrane signals and downstream transcription targets, the relative quantity of the intracellualr mediator (SMAD4) becomes critical'. Thus, the lack of gross and histologic abnormalities reported in *smad4*-deficient mice not also deficient in *Apc* (11) may be due to their having a *smad4* defect which allows somewhat more production of Smad4 than does the defect produced by Xu et al. The breeding stock was the same (Black Swiss), tending to minimize a systematic difference in one or more other genes affecting hyperplasia and neoplasia via interactions between their products and Smad4.

The extensive invasion and destruction of bone that occurred in three of the four squamous cell carcinomas that developed in the head and neck region suggest that these may have arisen within the bone. The observed continuity with skin or oral epithelium then would be the result of perforation of the buccal or lingual plate and collision with overlying epithelium. However, no continuity with odontogenic epithelium was seen, and there were extensive hyperplasia and dysplasia of the overlying oral or skin epithelium well beyond the areas of continuity with intrabony tumor. In addition, human primary intraosseous squamous cell carcinoma of the jawbones is rare (26), while oral squamous cell carcinoma frequently invades the jawbones, particularly the posterior mandible (27).

The significance of the abscesses and focal collections of lymphocyetes observed here is not clear, but it may be related to impaired TGF-β signaling. Smad3-deficient mice have impaired mucosal immunity and develop lymphocytic inflammation and abscesses (28), and the lacrimal and salivary glands of TGF-β1-deficient mice are progressively destroyed by a Sjögren's-syndrome like invasion of lymphocytes (29).

Smad4$^{+/-}$/*elf*$^{+/-}$ *mice*

Gastrointestinal hyperplasia was more advanced, involved much more of the mucosa, and had more and larger polyps, in all six *smad4*$^{+/-}$/*elf*$^{+/-}$ mice, compared with the eight *smad4*$^{+/-}$ mice in the same age range of 8–9 months. Indeed, a similar extent of hyperplasia and polyposis was found only in *smad4*$^{+/-}$ mice older than 17 months.

The hypertrophy of the submandibular salivary glands in the males occurred via an orderly hyperplasia of all cell types. It is unlikley that the hyperplasia was due to increased secretion of testosterone, as this would have resulted in a disproportionate increase in the androgen-dependent convoluted granular tubules (30). In this preliminary study, gland weights were not obtained, and thus the enlargement was not quantified. It is possible that a less overt enlargement of submandibular glands occurred in the females. In future studies, it might be useful to compare the weights and histometrically determined proportions of cell types of the submandibular and other major salivary glands of *smad4*$^{+/-}$/*elf*$^{+/-}$ mice with those of wild-type, *elf*$^{+/-}$ and *smad4*$^{+/-}$ mice.

Although only one malignancy was seen in the *smad4*$^{+/-}$/*elf*$^{+/-}$ mice, the sample consisted of six comparatively young mice. As noted previously, *Apc*-deficient mice developed numerous intestinal polyps which remained benign, while many of the polyps of *smad4*/*Apc* compound mutants developed into adenocarcinomas (11). Consequently, it was anticipated that, as a larger sample of *smad4*$^{+/-}$/*elf*$^{+/-}$ mice with a wider spectrum of ages became available, they would develop an incidence and variety of malignancies that may surpass those of the *smad4*$^{+/-}$ mice. This indeed has begun to occur. In addition, as previously mentioned, some of the *elf*$^{+/-}$ mice older than 1 year have begun to develop cancers.

On the value of smad4$^{+/-}$ *and smad4*$^{+/-}$/*elf*$^{+/-}$ *mutant mice in the study of neoplasia*

Production of mice in which one or both alleles of a gene are missing or otherwise inactivated is an excellent means to study the effects of the absence or deficiency of the products of that gene. Recently the National Cancer Institute's Mouse Models of Human Cancers Consortium has published a group of articles portraying the types of cancer that have occurred in mice as a result of such genetic as well as other manipulations (31). Here, we have presented a similarly straightforward documentation of the occurrence of hyperplasia and neoplasia in a much broader range of tissues in *smad4*$^{+/-}$ mice than had been previously observed, and in a new hybrid of *elf*- and *smad4*-deficient mice. Further investigation is underway into the roles of these and other genes in neoplasia via assessments of gene expression in the neoplasms that develop in these mice and in hybrids of *elf* and smads 2, 3 and 4 with mice deficient in genes controlling cell cycling, such as p53 and cdk4.

References

1. Roberts AB, Sporn MB. The transforming growth factor-βs. In: Sporn MB, Roberts AB, eds. *Peptide growth factors and their receptors*, Part I, Vol. **95**. Berlin: Springer-Verlag, 1990; 419–72.

2. ten Dijke P, Goumans M-J, Itoh F, Itoh S. Regulation of cell proliferation by Smad proteins. *J Cell Physiol* 2002; **191**: 1–16.
3. Varga J. Scleroderma and Smads. Dysfunctional Smad family dynamics culminating in fibrosis. *Arthritis Rheum* 2002; **46**: 1703–13.
4. Salovaara R, Roth S, Loukola A, et al. Frequent loss of SMAD4/DCP4 protein in colorectal cancers. *J Clin Pathol* 2002; **55**: 385–8.
5. Biankin AV, Morey AL, Lee C-S, et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. *J Clin Oncol* 2002; **20**: 4531–42.
6. Schutte M, Hruban RH, Hedrick L, et al. *DPC4* gene in various tumor types. *Cancer Res* 1996; **56**: 2527–30.
7. Boulay J-L, Mild G, Lowy A, et al. SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer. *Br J Cancer* 2002; **87**: 630–4.
8. Torbenson M, Marinopoulos S, Dang DT, et al. Smad4 overexpression in hepatocellular carcinoma is strongly associated with transforming growth factor beta II receptor immunolabeling. *Hum Pathol* 2002; **33**: 871–6.
9. Yang X, LI C, Xu X, Deng C. The tumor suppressor SMAD4/DPC4 is essential for epiblast proliferation and mesoderm induction in mice. *Proc Natl Acad Sci U S A* 1998; **95**: 3667–72.
10. Xu X, Brodie SG, Yang X, et al. Haploid loss of the tumor suppressor Smad4/Dpc4 inititiates gastric polyposis and cancer in mice. *Oncogene* 2000; **19**: 1868–74.
11. Takaku K, Oshima M, Miyoshi H, Matsui M, Seldin MF, Taketo MM. Intestinal tumorigenesis in compound mutant mice of both *Dpc4* (*Smad4*) and *Apc* genes. *Cell* 1998; **92**: 645–56.
12. Goodman SR, Zagon IS, Whitfield CF, et al. A spectrin-like protein from mouse brain membranes: phosphorylation of the 250,000-dalton subunit. *Am J Physiol* 1984; **247**: C61–73.
13. Mishra L, Voyles N, Bankert L, Mishra B, Mezey E, Gearhart J. ELF, a beta-spectrin, belongs to a set of novel markers which define early mouse liver development. *Int J Dev Biol* 1998; **41**: 747–50.
14. Mishra L, Cai T, Monga SPS, Mishra B. Elf3 encodes a novel 200-kD B-spectrin: role in liver development. *Oncogene* 1999; **18**: 353–64.
15. Tang Y, Katuri V, Iqbal S, et al. ELF a beta-spectrin is a neuronal precursor cell marker in developing mammalian brain; structure and organization of the elf/beta-G spectrin gene. *Oncogene* 2002; **34**: 5255–67.
16. Tang Y, Katuri V, Dillner A, Mishra B, Deng CX, Mishra L. Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. *Science* 2003; **299**: 574–7.
17. Little CC, Snell GD, Bittner JJ, et al. *Biology of the laboratory mouse*. New York: Dover Publications, 1941; 117–8.
18. Ham AW. *Histology*, 7th edn. Philadelphia: Lippincott, 1974; 654–8.
19. Lange D, Persson U, Wollina U, et al. Expression of TGF-beta related Smad proteins in human epithelial skin tumors. *Int J Oncol* 1999; **14**: 1049–56.
20. Natsugoe S, Xiangming C, Matsumoto M, et al. Smad4 and transforming growth factor beta1 expression in patients with squamous cell carcinoma of the esophagus. *Clin Cancer Res* 2002; **8**: 1838–42.
21. Fukuchi M, Masuda N, Miyazaki T, et al. Decreased Smad4 expression in the transforming growth factor-beta signaling pathway during progression of esophageal squamous cell carcinoma. *Cancer* 2002; **95**: 737–43.
22. Kim SK, Fan Y, Papadimitrakopoulou V, et al. DPC4, a candidiate tumor suppressor gene, is altered infrequently in head and neck squamous cell carcinoma. *Cancer Res* 1996; **56**: 2519–21.
23. Osawa H, Shitara Y, Shoji H, et al. Mutation analysis of transforming growth factor beta type II receptor, Smad2, Smad 3 and Smad4 in esophageal squamous cell carcinoma. *Int J Oncol* 2000; **17**: 723–8.
24. Logullo AF, Nonogaki S, Miguel RE, et al. Transforming growth factor β1 (TGFβ1) expression in head and neck squamous cell carcinoma patients as related to prognosis. *J Oral Pathol Med* 2003; **32**: 139–45.
25. Paterson IC, Davies M, Stone A, et al. TGF-β1 acts as a tumor suppressor of human malignant keratinocytes independently of Smad4 expression and ligand-induced G_1 arrest. *Oncogene* 2002; **21**: 1616–24.
26. Thomas G, Pandey M, Mathew A, et al. Primary intraosseous carcinoma of the jaw: pooled analysis of world literature and report of two new cases. *Int J Oral Maxillofac Surg* 2001; **30**: 349–55.
27. Brown JS, Lowe D, Kalavrezos N, D'Souza J, Magennis P, Woolgar J. Patterns of invasion and routes of tumor entry into the mandible by oral squamous carcinoma. *Head Neck* 2002; **24**: 370–83.
28. Yang X, Letterio JJ, Lechleider RJ, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999; **18**: 1280–91.
29. McCartney-Francis NL, Mizel DE, Redman RS, et al. Autoimmune Sjögren's-like lesions in salivary glands of TGF-β1 deficient mice are inhibited by adhesion-blocking peptides. *J Immunol* 1996; **157**: 1306–12.
30. Gresik EW. The granular convoluted tubule (GCT) cell of rodent submandibular glands. *Microsc Res Tech* 1994; **27**: 1–24.
31. Galvez JJ, Cardiff RD, Munn RJ, Borokowsky AD. Overview. Mouse models of human cancers (part 2). *Comp Med* 2004; **54**: 13–5.

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