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*Review*

# Ets transcription factors in intestinal morphogenesis, homeostasis and disease

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**Summary.** Ets transcription factors comprise a large family of sequence-specific regulators of gene expression with important and diverse roles in development and disease. Most Ets family members are expressed in the developing and/or mature intestine, frequently in a compartment-specific and temporally dynamic manner. However, with the exception of the highly expressed Elf3, involved in embryonic epithelial differentiation, little is known about Ets functions in intestinal development and homeostasis. Ets factors show altered expression in colon cancer, where they regulate pathways relevant to tumor progression. Ets factors also likely act as important modifiers of non-neoplastic intestinal disease by regulating pathways relevant to tissue injury and repair. Despite a large body of published work on Ets biology, much remains to be learned about the precise functions of this large and diverse gene family in intestinal morphogenesis, homeostasis, and both neoplastic and non-neoplastic pathology.

**Key words:** Ets, Transcription factor, Intestine, Morphogenesis, Homeostasis, Cancer

## Ets factors – Introduction

Ets factors comprise a large family of transcription factors related to each other by a conserved DNA-binding domain (DBD), the Ets domain. Found in metazoans, the number of individual Ets factors increases with the complexity of the organism, up to a total of 27 in humans (Gutierrez-Hartmann et al., 2007). Ets factors frequently function as mediators of extracellular signaling pathways (Wasylyk et al., 1998; Sharrocks, 2001; Oikawa and Yamada, 2003). As sequence-specific DNA-binding proteins, Ets factors interact with a core GGA(A/T) DNA sequence via the conserved Ets DBD, a winged helix-turn-helix structural motif (Graves and Petersen, 1998; Sharrocks, 2001; Oikawa and Yamada, 2003). Individual Ets factors regulate promoter activity directly via intrinsic activation or, less commonly, repression domains, or indirectly through interactions with other transcription-modulating proteins (Graves and Petersen, 1998; Wasylyk et al., 1998; Sharrocks, 2001; Oikawa and Yamada, 2003). Occasionally, some Ets factors may either activate or repress transcription depending on the precise promoter context (Oikawa and Yamada, 2003). Ets factors modulate the expression of a variety of genes involved in diverse cellular processes, including cell proliferation, differentiation, apoptosis, and cell-cell/cell-matrix interactions (Sementchenko and Watson, 2000). Many aspects of Ets biology have been the subject of recent reviews (Sharrocks, 2001; Oikawa and Yamada, 2003; Hsu et al., 2004; Seth and Watson, 2005; Gutierrez-Hartmann et al., 2007). This review will focus on Ets factors in the morphogenesis, homeostasis and disease of the intestinal tract.

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## Ets factors in intestinal morphogenesis and homeostasis

Mouse genetic studies have revealed unique Ets functions in a variety of biological processes. Gene inactivation ("knock-out") studies have shown Ets factors to perform essential functions in: hematopoiesis and immune function (Ets1, Elf4, Fli1, Tel, Spi1, SpiB, GABPα); lymph/angiogenesis (Tel, Elk3); neurogenesis and neuromuscular function (Pea3, Erm, Er81, GABPα); spermatogenesis (Erm); development of extraembryonic tissues and early embryonic development (Ets2, Elf5, GABPα) (Maroulakou and Bowe, 2000; Lacorazza et al., 2002; Livet et al., 2002; Oikawa and Yamada, 2003; Ristevski et al., 2004; Rosmarin et al., 2004; Chen et al., 2005; Donnison et al., 2005; Zhou et al., 2005; Georgiades and Rossant, 2006; Hippenmeyer et al., 2007; O'Leary et al., 2007). As multiple Ets factors tend

to be expressed in the same cell (Galang et al., 2004; Hollenhorst et al., 2004) and there appears to be substantial overlap in Ets DNA-binding specificity (Graves and Petersen, 1998), gene inactivation experiments may not reveal all Ets functions *in vivo*, due to potential compensatory activity by other co-expressed Ets factors. Indeed, tissue-specific expression of genetically modified Ets factors designed to block such compensatory activity has uncovered a number of Ets functions (de Kerchove D'Exaerde et al., 2002; Paratore et al., 2002; Parkinson et al., 2002; Theveneau et al., 2007), including pulmonary airway morphogenesis not seen in Ets knock-out mice (Liu et al., 2003). As noted above, Ets factors frequently act as mediators of cell-cell signaling pathways, and this is true in development, where Ets factors often function as nuclear effectors of fibroblast growth factor (FGF) signaling (Raible and Brand, 2001; Roehl and Nusslein-Volhard, 2001; Kawachi et al., 2002; Bertrand et al., 2003; Liu et al., 2003; Brent and Tabin, 2004).

A number of approaches have revealed widespread expression of Ets family members in the developing and mature mammalian intestine (Table 1, Fig. 1). Global expression profiling approaches performed on whole tissue have shown the expression of most Ets factors in the developing small intestine, many with temporally dynamic patterns of expression (Lepourcelet et al., 2005; Choi et al., 2006). By RT-PCR analysis, approximately one third of Ets factors analyzed showed changes in expression levels from mouse embryonic day 11 to 17, a period of dynamic tissue remodeling in the intestine (Choi et al., 2006). Global gene expression profiling of fractionated tissue and in-situ hybridization analysis have further demonstrated tissue compartment-specific expression in the intestine for many Ets factors (Kola et al., 1993; Maroulakou et al., 1994; Chotteau-Lelievre et al., 1997; Oettgen et al., 1997; Maroulakou and Bowe, 2000; Vlaeminck-Guillem et al., 2000; Li et al., 2007). In the developing mouse small intestine, Pea3, Elf1, Elf3, Ehf, Ets2 and Erf are predominantly epithelial; Er81, Elk3, Elf2, Ets1, Erg and Fli1 are predominantly non-epithelial; and Erm appears to be expressed in both

**Table 1.** Physiologic Ets transcription factor expression in mammalian intestine, and altered expression in colon cancer.

| Ets factor | Developmental expression (mouse)[1] | Relative expression (human adult sm int; colon; HCT-116 cells)[2] | Altered expression in colon carcinoma[3] |
|---|---|---|---|
| ER81/ETV1* | WTE (SAGE); nE (ISH; ma, 10.3) | 2; 2; 3 | Up (WTE: RT) |
| PEA3/ETV4/E1AF* | WTE (SAGE); E (ISH) | 2; <1; 17 | Up (WTE: RT, ma [2]) |
| ERM/ETV5* | WTE (SAGE; RT); E/nE (ISH); nE (ma, 8.4) | 1; 1; 32 | Up (WTE: RT) |
| ELK1* | WTE (SAGE; RT) | 5; 5; 19 | |
| ELK3/NET/SAP2/ERP* | WTE (SAGE; RT); nE (ma, 53.0) | 6; 3; 26 | |
| ELK4/SAP1 | WTE (RT) | 14; 18; 16 | |
| ELF1 | WTE (SAGE; RT); E (ISH) | 4; 4; 4 | Up (WTE: ma [1]) |
| ELF4/MEF/ELFR* | WTE (SAGE; RT) | 7; 13; 9 | |
| ELF2/NERF | WTE (SAGE); nE (ma, 2.2); | 6; 7; 15 | |
| ELF3/ESE1/ESX/ERT/JEN* | WTE (SAGE); E (ISH; ma, 5.7) | 115; 449; 84 | |
| ELF5/ESE2* | WTE (RT) | <1; <1; <1 | |
| EHF/ESE3* | WTE (SAGE); E (ma, 11.1) | 47; 183; 38 | |
| SPI1/PU1* | | 16; 11; <1 | |
| SPIB* | | 3; 3; <1 | |
| SPIC | WTE (RT) | <1; <1; <1 | |
| TEL/ETV6* | WTE (SAGE; RT) | 11; 18; 10 | Down (WTE: ma [1]) |
| TEL2/TREF | NA | 4; 5; 1 | |
| PDEF/ESF/PSE | WTE (RT) | 3; 17; <1 | |
| GABPα/E4TF1* | WTE (SAGE) | 12; 10; 38 | |
| ETV2/ER71 | WTE (RT) | 2; 2; 4 | |
| ETS1* | WTE (SAGE; RT); nE (ISH; ma, 104.3) | 24; 18; 5 | Up (IHC) |
| ETS2* | WTE (SAGE; RT); E (ISH; ma, 2.0) | 68; 50; 25 | Up (IHC; WTE: ma [1]) |
| ERF | WTE (SAGE, RT); E (ma, 2.9) | 3; 2; 3 | |
| ETV3/PE1 | WTE (SAGE; RT) | 11; 7; 12 | |
| ERG | nE (ISH; ma, 9.4) | 3; 2; <1 | |
| FLI1/ERGB* | WTE (SAGE; RT); nE (ma, 42.4) | 4; 2; <1 | Down (WTE: ma [1]) |
| FEV | WTE (RT) | 5; 3; <1 | |

[1] WTE: expression analyzed in whole tissue extract (SAGE [serial analysis of gene expression] of mouse embryonic small intestine from (Lepourcelet et al., 2005), RT: [reverse transcriptase polymerase chain reaction] analysis of mouse embryonic small intestine from (Choi et al., 2006)). Predominantly epithelial (E) or non-epithelial (nE) expression as determined by *in-situ* hybridization analysis (ISH; see text for references) and/or microarray (ma) analysis of chemically fractionated E18.5 mouse small intestine ((Li et al., 2007); numerical value is fold-enrichment in epithelial or non-epithelial compartment). [2] Number of mRNA molecules per $2x10^6$ molecules of 18S rRNA, as determined by quantitative RT-PCR analysis of adult human whole tissue extracts (Hollenhorst et al., 2004); sm int: small intestine; HCT-116 cells: colon cancer cell line. [3] WTE: expression analyzed in whole tissue extract; RT: semi-quantitative reverse transcriptase polymerase chain reaction; IHC: immunohistochemical analysis; ma: microarray analysis (tumor vs normal data from Oncomine™ Research database, www.oncomine.org, searched at $p<0.001$; number in brackets: number of studies showing this change). See text for additional detail, including references. *genetically inactivated (knocked-out) in mouse; NA: not found in mouse.

compartments (Table 1, Fig. 1). In the limited data available from the adult intestine, Elf3 expression remains epithelial and Ets1 remains non-epithelial (Table 1, Fig. 1). The resolution of such studies has thus far been largely limited to epithelium versus non-epithelial tissue. Although the latter is frequently referred to as "mesenchyme" or "stroma", it is a complex tissue compartment composed of multiple different cell types. Thus, higher resolution expression studies will be required to determine which Ets factors are truly "mesenchymal/stromal", and which are expressed in smooth muscle, vascular, neural, and immune/inflammatory cells (both those resident to the intestine and those incidentally transiting intestinal vasculature). In the adult intestine, expression of essentially all Ets transcription factors can be demonstrated at the whole tissue level, but transcript levels vary widely (Table 1). Elf3 and Ehf, two epithelial-specific Ets factors are expressed at high levels. Also showing relatively high-level expression are Ets1 and Ets2. In contrast, some Ets factors, notably members of the developmentally expressed PEA3 subfamily (Pea3, Erm and Er81) are expressed at relatively low levels in the adult. It remains to be determined whether low-level expression reflects a lesser requirement or essential, but spatially restricted, function. Finally, also at the whole tissue level, there appear to be some differences in Ets expression levels between the small intestine and colon. Notably, in the adult, the epithelial-specific Ets factors (Elf3, Elf5, Ehf and PDEF), as well as members of the PEA3 subfamily (Pea3, Erm and Er81), appear to be expressed at higher levels in the colon (Chotteau-Lelievre et al., 1997; Hollenhorst et al., 2004). In sum, available expression data strongly suggest important and potentially specific functions for Ets factors in the developing and mature intestine.

Of the nearly two-thirds of Ets factors genetically inactivated in the mouse thus far (Table 1), only one, the epithelial-specific Ets factor Elf3, has been reported to have an intestinal phenotype (Ng et al., 2002). Elf3$^{-/-}$ embryos manifest delayed and impaired villus morphogenesis, impaired enterocyte differentiation and altered microvillus structure, and altered goblet cell differentiation. Elf3$^{-/-}$ newborn animals exhibit diminished weight gain, and adults develop a "wasting" phenotype characterized by weight loss and diarrhea. Interestingly, the Elf3$^{-/-}$ embryonic phenotype is associated with diminished epithelial expression of transforming growth factor ß type II receptor (TGFßRII), and both the impaired enterocyte and goblet cell differentiation can be rescued by transgenic TGFßRII expression in the intestinal epithelium (Flentjar et al., 2007). Thus, the critical role of Elf3 in epithelial differentiation in the developing intestine appears to be the facilitation of TGFß signaling (Fig. 2). The role of Elf3 in regulation of TGFßRII expression in intestinal epithelium likely involves direct stimulation of TGFßRII promoter activity (Choi et al., 1998). Other Ets factors have been shown to be capable of regulating the TGFßRII promoter, with different, and often context-dependent, effects (Kopp et al., 2004). It will be interesting to see if other co-expressed Ets factors have a role in modulating TGFßRII expression in intestinal epithelium, and thus its differentiation.

Given the apparent ability of Ets factors to compensate for one another genetically, a possible reason for the paucity of intestinal phenotypes in other Ets knock-out animals is compensatory activity by Elf3. At the same time, the phenotype of Elf3 knock-out animals may not reflect the full spectrum of Elf3 function *in vivo* due to possible compensatory activity of other Ets factors, such as the closely related, and also highly expressed, Ehf. More precise analysis of the spatiotemporal patterns of Ets expression in the intestine, detailed analysis of intestinal development and homeostasis in individual, and potentially compound, Ets knock-out animals, as well as use of genetically modified Ets factors designed to overcome Ets compensation *in vivo* will be needed to fully characterize the functions of the many Ets factors expressed in the intestine. Of particular interest will be the other epithelial-specific Ets factors (Elf5, Ehf and PDEF), and other Ets factors implicated in morphogenic/homeostatic processes in other tissues, including members of the PEA3 subfamily (Pea3, Erm and Er81), Ets2 and GABPα. Interestingly, FGF signaling has been

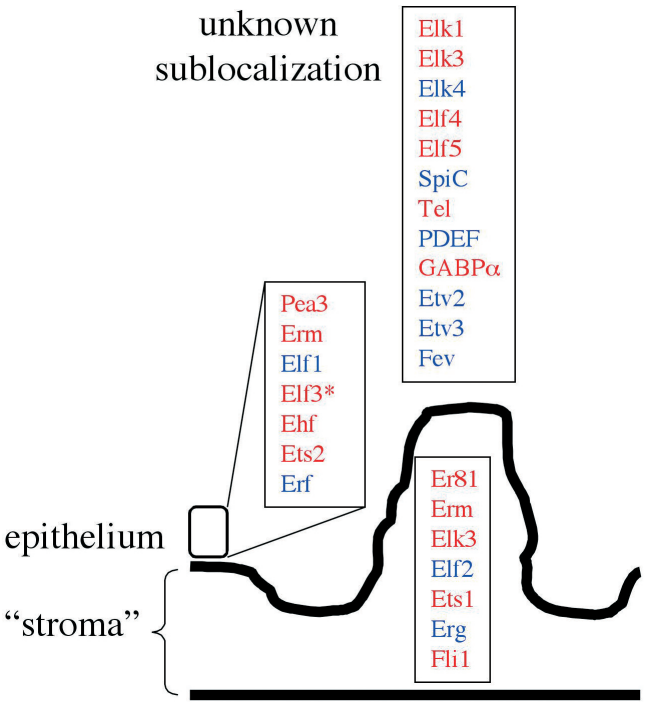


**Fig. 1.** Ets expression in the developing mouse intestine. Ets factors with demonstrated developmental expression in the epithelium, non-epithelial tissue ("stroma"), or in whole tissue (unknown sublocalization). Red: genetically inactivated (knocked out) in mouse; asterisk (*): intestinal phenotype reported in knock-out mice.

demonstrated to be required for cecal morphogenesis in the mouse (Burns et al., 2004; Zhang et al., 2006). It remains to be determined if Ets factors are involved in this process, as they are in other FGF-mediated morphogenic processes.

### Ets factors in intestinal epithelial neoplasia

A number of different Ets factors have been shown to be misexpressed or overexpressed in human malignancies (Kurpios et al., 2003; Oikawa and Yamada, 2003; Seth and Watson, 2005; Gutierrez-Hartmann et al., 2007). In hematologic malignancies, Ets overexpression appears to be driven at least in part by gene amplification (Rovigatti et al., 1986; Santoro et al., 1992; Baldus et al., 2004; Poppe et al., 2004). Gene amplification has also been demonstrated for Elf3 in breast cancer (Chang et al., 1997), and in prostate cancer a number of Ets factors become overexpressed by a chromosomal translocation-driven mechanism (Tomlins et al., 2005). The mechanisms governing Ets overexpression in other solid tumors remain to be defined. Much also remains to be learned about the precise downstream pathways mediating Ets tumor-modifying effects. Plausible candidate mechanisms, including effects on proliferation, survival, migration, invasion and angiogenesis, and targets, including extracellular matrix components and modifying enzymes, have been postulated based on tissue culture models and other studies, but largely remain to be demonstrated *in vivo* (Coletta et al., 2004; Hsu et al., 2004; Seth and Watson, 2005). *In vivo*, epithelial Pea3 and stromal Ets2 have been shown to promote mammary epithelial tumor growth in mouse models (Shepherd et al., 2001; Man et al., 2003). The stromal tumor-promoting effects of Ets2 act downstream of VEGF signaling, require MAP kinase-mediated phosphorylation of Ets2, and regulate downstream MMP expression (Man et al., 2003).

A number of Ets factors have been shown to be misexpressed/ overexpressed in colon carcinoma (Table 1). Increased Ets1 and Ets2 expression in tumor cells correlates with adenoma to carcinoma progression, with Ets1 expression also correlating with carcinoma depth of invasion, lymphovascular invasion and metastasis (Nakayama et al., 2001; Ito et al., 2002). Ets1 is also expressed in tumor stroma, where it correlates with adenoma to carcinoma progression, lung metastasis, stromal expression of the matrix metalloproteases MMP-1 and MMP-9, and vascular expression of integrin ß3 (Sato and Miwa, 2002; Behrens et al., 2003). Expression of Ets1, as well as possibly Ets2, in non-epithelial tissue compartments may explain the observed lower expression of these Ets factors in some colon cancer cell lines in comparison to unfractionated whole colon tissue (Hollenhorst et al., 2004). Ets2 is positively regulated by active Wnt signaling (van de Wetering et al., 2002), which may represent one mechanism for Ets everexpression in tumors.

Overexpression of Pea3, but not Erm, Er81, Ets1 or Ets2, predicts poor survival in colon carcinoma, and is associated with increased expression of MMP-1 and matrilysin/MMP-7 (Horiuchi et al., 2003). Pea3, Erm and Er81 expression levels are also increased in colon cancer cell lines relative to normal tissue (Crawford et al., 2001; Hollenhorst et al., 2004). In cultured colon cancer cells, Pea3 is required for MMP-1 and matrilysin/MMP-7 expression and invasive behavior (Horiuchi et al., 2003). Pea3, as well as the other members of the PEA3 Ets subfamily (Erm and Er81), cooperate with the ß-catenin/TCF complex and c-Jun to stimulate matrilysin promoter activity (Crawford et al., 2001). Matrilysin functions as a tumor promoter in vivo (Witty et al., 1994; Wilson et al., 1997). Thus, stimulation of matrilysin expression likely represents an important mechanism of colon cancer promotion by Pea3 (Fig. 3). Other Ets-regulated tumor-promoting genes in colon cancer include Cox-2 and osteopontin (Liu et al., 2004; Wai et al., 2006). In addition, Ets factors may be involved in the regulation of cyclinD1 expression by ß-catenin (Tetsu and McCormick, 1999).

Studies of other malignancies have suggested tumor suppressor functions for some Ets factors, including the epithelial-specific Ehf and PDEF (Gu et al., 2007; Turner et al., 2007a,b; Cangemi et al., 2008), and Elf4 (Seki et al., 2002). Expression of Elf3, Ehf and PDEF, three of the four epithelial-specific Ets factors, is several-fold lower in the HCT-116 colon cancer cell line compared to unfractionated whole colon tissue (Table 1), and the LoVo colon cancer cell line is also reported to lack detectable PDEF protein (Turner et al., 2007b). As these Ets factors are expressed predominantly, if not exclusively, in epithelia (Feldman et al., 2003), these differences are not likely to be accounted for by expression in the non-epithelial tissue compartment of

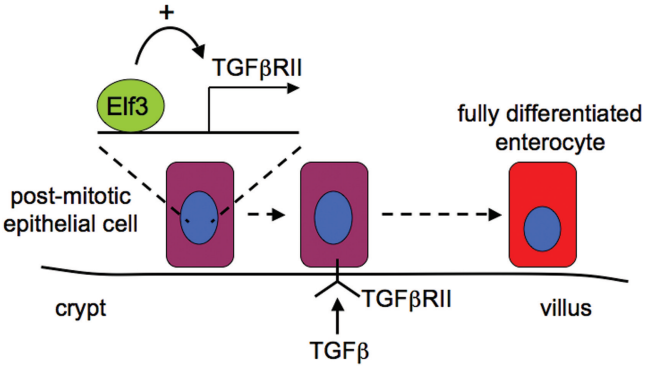


**Fig. 2.** Elf3 in developmental enterocyte differentiation. Elf3, expressed in developing intestinal epithelium, promotes epithelial expression of the TGFß type II receptor (TGFßRII), probably by directly stimulating TGFßRII promoter acivity. This presumably permits an epithelial response to the differentiating effects of TGFß, thus promoting epithelial differentiation along the enterocyte lineage. The same pathway also appears to regulate goblet cell terminal differentiation (see text).

normal tissue. It remains to be determined whether the epithelial-specific Ets factors, as well as possibly others, have tumor suppressor functions in colon cancer, possibly in part through regulation of TGFßRII expression. Furthermore, while overexpressed Ets2 behaves as a tumor-promoter in multiple tumor types (Seth and Watson, 2005), physiologic or near-physiologic gene dosage of Ets2 has recently been shown to have a tumor "repressive" effect in a mouse model of colon cancer (Sussan et al., 2008). This suggests the interesting possibility that Ets factors may exert different, including opposing, tumor-modifying effects at normal/near-normal versus aberrantly high expression levels, due to differential binding and/or modulation of gene regulatory regions.

Finally, global gene expression profiling studies of unfractionated tumor and normal tissue confirm increased expression of Ets2 and Pea3 in colon cancer, and reveal additional alterations in Ets expression, including increased Elf1 and decreased Tel and Fli1 (Table 1). Furthermore, a study profiling tissue adjacent to sporadic colon carcinoma identified increased Erm, Er81 and Net expression, and decreased Ehf, Elf1 and Elf4 expression, relative to tissue from individuals without cancer (Hong et al., 2007), suggesting possible functions for these Ets factors in colon cancer initiation and/or early progression. Taken together, the above data implicate Ets factors in many aspects of colon carcinoma initiation and/or progression, but much remains to be learned about their causal roles *in vivo*, as well as the precise mechanisms by which they effect these roles.

## Ets factors in non-neoplastic disease of the intestine

Far less is known about Ets factor function(s) in non-neoplastic disease, but, as in neoplasia, Ets factors appear to be important disease modifiers. Ets1 exerts an anti-fibrogenic effect in fibroblasts, antagonizing the effects of TGFß (Knittel et al., 1999; Czuwara-Ladykowska et al., 2002), and Ets1 knock-out mice exhibit features of autoimmune disease in multiple organs (Wang et al., 2005). Additionally, 3' polymorphisms of Ets1 are associated with different clinical manifestations of systemic lupus erythematosus (Sullivan et al., 2000). Ets2 and Fli1 overexpression has been observed in a number of inflammatory/autoimmune diseases (Trojanowska, 2000), and, interestingly, the normally epithelially restricted Elf3 becomes misexpressed in non-epithelial cells under conditions of inflammation (Rudders et al., 2001; Grall et al., 2003; Brown et al., 2004). Lastly, the Ets factor Net is required for VEGF-mediated angiogenesis in a mouse model of cutaneous wound healing (Zheng et al., 2003). Ets factors have been little studied in non-neoplastic intestinal disease, although quantitative alterations in Ets1 mRNA and protein have been observed in inflammatory bowel disease (Konno et al., 2004). Further, Elf3 has been shown to regulate the expression of the pro-inflammatory cytokine MIP-3α in enterocytes (Kwon et al., 2003). Global gene expression profiling of intestinal tissue from individuals with inflammatory bowel disease (IBD) compared to controls has not revealed significant alterations in the expression levels of Ets factors other than Ets1 at the whole tissue level (Hughes, 2005). However, given the complexity of tissue pathology in IBD, this does not exclude small and/or tissue subcompartment-restricted, but mechanistically significant, Ets expression changes. Similarly, it also does not exclude potentially important functions for physiologic levels of Ets during tissue injury and/or healing. Overall, given the important functions of Ets factors in epithelial, stromal, vascular and immune/inflammatory biology, it is likely that they have important roles in non-neoplastic intestinal disease, including IBD, but these roles largely remain to be identified and characterized.

## Summary and perspectives

Ets transcription factors are widely expressed in the developing and mature intestine. Multiple approaches demonstrate tissue compartment and subcompartment-specific, and in some cases temporally dynamic, expression patterns for a number of Ets factors. This suggests specific functions for different Ets in intestinal morphogenesis and homeostasis, thus far demonstrated *in vivo* for only one Ets factor, Elf3. Elf3 controls intestinal epithelial differentiation during development by regulating the expression of TGFßRII in epithelial cells. Rigorous analysis of intestinal development, homeostasis and pathology in individual Ets knock-out animals, combined with creative genetic approaches, will be required to learn more about Ets functions in the

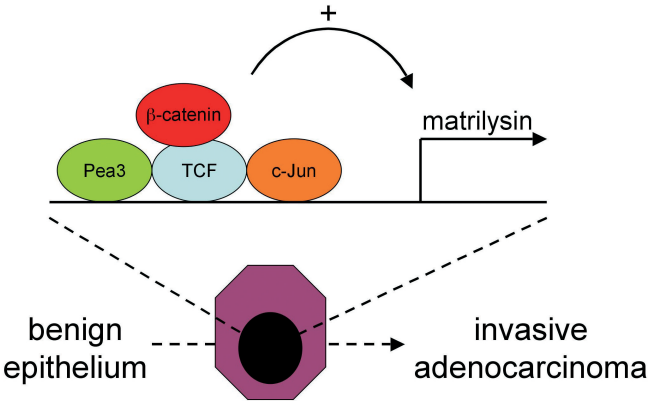


**Fig. 3.** Pea3 in colon cancer. Pea3 is overexpressed in tumor cells where it cooperates with the ß-catenin/TCF complex and c-Jun to stimulate the expression of the tumor promoter matrilysin. Matrilysin is expressed from early on in the adenoma-carcinoma sequence and may have multiple tumorigenesis-modifying functions (Witty et al., 1994; Takeuchi et al., 1997; Wilson et al., 1997). Other Ets-regulated tumor-promoting targets in colon cancer include Cox-2 and osteopontin, and Ets factors in the stromal, vascular and other surrounding tissue compartments likely also have tumor-modifying functions (see text).

intestine in vivo. Expression and in vitro function studies suggest tumor-promoting roles for a number of Ets factors in colon cancer. It remains to be determined whether some Ets factors, including epithelial-specific Ets, function as tumor suppressors in colon cancer, as they appear to do in other malignancies. Given the important functions of Ets factors in epithelial, stromal, vascular and immune/inflammatory biology, it is likely that Ets factors have important roles in non-neoplastic diseases of the intestine, including inflammatory bowel disease, but, at present, these roles remain to be identified.

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