

Invited Review

EGF receptor signaling in prostate morphogenesis and tumorigenesis

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Summary. The growth and differentiation of the prostate gland are largely dependent on extracellular signaling factors. In addition to androgens, many polypeptide growth factors function through autocrine or paracrine networks. The paracrine interaction between stromal and epithelial cells is critical for androgen regulation, morphogenesis, epithelial cell proliferation, and secretory differentiation. Efforts to identify the essential growth factors and studies on their effects have been prompted by the fact that prostate cells in culture need substances other than androgens for proliferation. In this context, transforming growth factor- α and epidermal growth factor, among others, have been studied extensively. Recent advances have suggested that these EGF receptor (EGFR) ligands play roles not only during glandular development but also during neoplastic transformation and tumor progression. The cell responses most relevant to the role of this receptor signaling are both mitogenesis and cell motility. The aim of the review is to provide an overview of current knowledge about EGFR and its ligands in the organogenesis and tumorigenesis of the prostate gland.

Key words: Organogenesis, Prostate carcinoma, Development, Signaling, Tumor Invasion

Prostate development

The prostate gland develops from the embryonic urogenital sinus. It can be first detected during midgestation (day 17 in mice and days 18-19 in rats) (Cunha et al., 1987) and does not reach adult form until adolescence (approximately day 30 in both mice and rats) (Sugimura et al., 1986; Hayashi et al., 1991). This growth is characterized by increased numbers of acini with more and more well-developed folding of the glandular elements (Sugimura et al., 1986; Hayashi et

al., 1991). The prostate pre- and postnatal development and adult maintenance of morphology and secretory functions are androgen dependent. In all stages of male development, androgens act upon specific intracellular androgen receptors which have been detected both biochemically and autoradiographically (Shannon and Cunha, 1983; Takeda et al., 1985). Ablation of fetal testes or chemical castration during prostate development results in the inhibition of the development of the prostate and other male accessory sexual glands and other male accessory sexual glands (for review see (Cunha et al., 1987)). Postnatally, prostatic development was greatly inhibited in experiments where neonatal mice or rats were castrated. The castration effect could be reversed by administration of testosterone (Price, 1936). The dependency of the adult prostate on androgens for the maintenance of morphology and functional activity is evident after castration. A remarkable reduction in prostatic size was shown in experiments performed on rats; prostatic epithelial cell apoptosis was observed after castration (Buchovsky et al., 1974; Isaacs, 1984). In addition, epithelial and stromal cells are lost disproportionately (reviewed by (Cunha et al., 1987)).

As observed in other organs containing an epithelial parenchyma, the development of the prostate is dependent upon the interaction between mesenchyme and epithelium. Work by Cunha and colleagues (Cunha et al., 1992) demonstrated that during prostatic fetal development, the mesenchyme and not the epithelium is the target site and mediator of androgen induced epithelial ductal morphogenesis and differentiation. This mediation of epithelial action is believed to result from androgens acting directly on stromal cells to elicit the synthesis of growth factors, and likely results from a linked epithelial-stromal-epithelial signaling loop described in the next section.

Prostate fluid contains the highest concentration of EGF in the human body (Marti et al., 1989). EGF and other EGF receptor (EGFR) activating factors (including TGF- α and amphiregulin) have been shown to be expressed in normal and neoplastic prostate cells (Eaton et al., 1988; Nishi et al., 1988; Connolly and Rose, 1989; Wilding et al., 1989; Liu et al., 1993). However, it is

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thought that these factors are secreted from the apical membrane into the prostatic fluid and would not be accessible to basolateral EGFR. Although levels of these factors were not shown to be increased by DHT in an androgen-independent cell line (Connolly and Rose, 1989); recent studies suggest androgens stimulate prostate proliferation of an androgen-responsive cell line by upregulating an paracrine stimulatory growth loop involving the EGFR on the basolateral face of prostate epithelium responding to stromally-produced transforming growth factor- α (TGF- α) (Liu et al., 1993). These data provide a central role for polypeptide factors in general, and EGF receptor activating factors in particular, in prostate growth and tumorigenesis (reviewed by (Story, 1991; Ware, 1993; Lalani et al., 1997)).

Growth factors and cognate receptors in the prostate

Four major growth factor families (insulin-like growth factor, epidermal growth factor, transforming growth factors and heparin binding growth factors/fibroblast growth factors) have mitogenic effects on prostate cells and prostate tissues *in vitro* and are present *in vivo*. Furthermore, receptors for these factors can be found on prostate epithelial cells (Burgess and Maciag, 1989; Carpenter and Cohen, 1990; Massague, 1990; Czech and Buxton, 1993). Based on these observations, these factors are presumed to function in the prostate gland (Table 1).

In contrast to classical endocrine hormones, growth factors are generally produced locally and have their effects within a local area. Some factors including platelet derived growth factors (PDGF) and transforming growth factor- α (TGF- α), may originate from a cellular component of the circulatory system, but are released locally. These locally acting factors have been categorized as autocrine or paracrine to describe their origin and target cells in local tissue (Sporn and Todaro, 1980). An autocrine factor both originates in one cell type and affects the same cell type while paracrine factors are those that originate in one cell type and affect another type of cells.

The failure to connect androgens directly to regulation of prostate growth at the molecular and cellular levels has led to the hypothesis that these

polypeptide growth factors and their receptors may be important mediators between blood borne androgens and prostate cell growth *in vivo*. A currently supported model for prostate epithelial responsiveness involves a double paracrine network (Fig. 1). In this model, the prostate epithelial cells, possessing 5- α -reductase, convert circulating testosterone to dihydrotestosterone which then signals the prostate stromal cells (fibroblasts) to secrete two critical growth factors – transforming growth factor- α (TGF- α) and keratinocyte growth factor (KGF or FGF-7). These factors, in turn, act upon the prostate epithelial cells. In this fashion the two major cell types of the prostate, epithelial and stromal, are maintained in balance. In this model, dys-synchrony of this loop would result in either BPH if the stromal or epithelial compartments grew preferentially, but in a controlled manner, or carcinoma if the epithelial cells grew unregulated.

EGF and TGF- α , two closely related mature polypeptides which consist of 53 and 50 amino acids, share about 35% sequence homology, and bind to and activate the same receptor (Ullrich et al., 1984), are produced in the prostate gland. EGF is produced primarily by the epithelial cells and is released into the prostatic fluid at very high concentrations. In fact, the prostatic fluid has the highest concentration of EGF in the body (Marti et al., 1989). In the normal prostate TGF- α appears to be secreted in low quantities by the stromal cells. One question that arises is how are the epithelial cells responsive to stromally-produced TGF- α but not autocrine EGF? Observations in prostate cells and extrapolation from other epithelial tissues would suggest that the EGFR are presented on the basolateral surfaces of the epithelial cells. Being below the tight junctions,

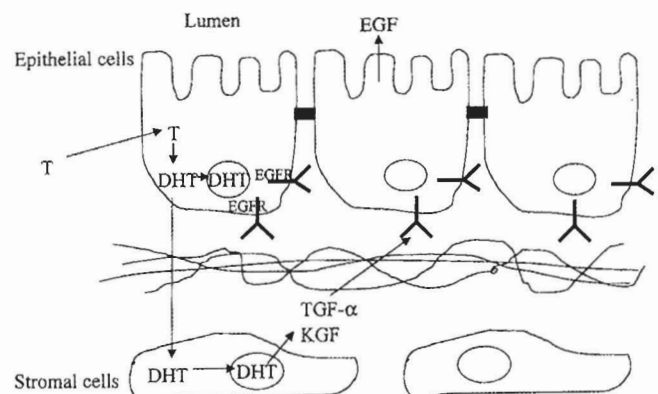


Fig. 1. Prostate epithelial and stromal cell cross-communication. The prostate gland functions as a single unit with numerous channels for communication between the epithelial and stromal compartments. Illustrated is the crosstalk which involves EGFR and its ligands in the physiological functioning of the prostate gland. T: testosterone derived from circulation; DHT: dihydrotestosterone from reduction (5- α -reductase-mediated) in the epithelial cells; KGF: FGF-7. The double bars represent tight junctions which separate the apical membrane compartment from the basolateral membrane compartment; only the latter present appreciable levels of EGFR.

Table 1. Major growth factor families acting in the prostate.

RECEPTOR	LIGANDS	REFERENCE
Epidermal growth factor receptor	EGF, TGF- α	Carpenter and Cohen, 1990
Transforming growth factor β receptors	TGF- β 1,2,3	Massague, 1990; Itoh, et al., 1998
Insulin and insulin like growth factor receptors	Insulin, IGF-I	Czech and Buxton, 1993
Fibroblast growth factor receptors	FGF-1,2,7	Burgess and Maciag, 1989; Yan, et al., 1993

these EGFR would not be accessible to the high levels of prostatic fluid EGF but only the low concentrations of stromal TGF- α . Such a situation would allow for rapid repair of any breaks in any epithelial monolayer, as the cells at the edge of the wound would now be stimulated by the mitogenic and motogenic EGF.

EGF Receptor signaling

The EGF receptor, which binds EGF, TGF- α and other specific ligands, is the prototypal receptor with intrinsic protein tyrosine kinase activity (Wells et al., 1998). In its mature form, it is a 170kDa transmembrane glycoprotein. Upon binding of ligand by its extracellular domain, the receptor homo- and hetero-aggregates with the closely related c-erbB receptors (Carraway and Cantley, 1994) and undergoes autophosphorylation of tyrosine residues. It has been shown that receptor autophosphorylation is the crucial event for signal transduction (Olivier et al., 1993). Phosphorylation of tyrosine residues creates docking sites for molecules containing Src homology (SH2) and phosphotyrosine binding (PTB) domains (Carpenter, 1992; Pelicci et al., 1992; Skolnik et al., 1993; Kavanaugh and Williams, 1994). These interactions with downstream molecules create signaling complexes which lead to pleiotropic responses by linking EGFR activation to a myriad of cytoplasmic signaling pathways.

EGFR activation also triggers cascades that attenuate its own signaling (Fig. 2). Upon ligand binding EGFR are internalized via clathrin-coated pits by specific adaptins that bind to multiple tight turn domains in the carboxy-terminus of the receptor (Chen et al., 1989; Sorkin and Carpenter, 1993) leading to degradation of occupied and active EGFR. In the face of an autocrine signaling loop, this ligand-induced degradation of EGFR

would give a false impression that EGFR are low or absent on cells; a situation which has confounded EGFR studies on isolated prostate epithelial and carcinoma cells. In addition, the receptor activity is attenuated by reflective PKC phosphorylation at a juxtamembrane threonine (threonine 654) (Welsh et al., 1991; Chen et al., 1996b), MAP kinase phosphorylation to other juxtamembrane amino acids (Morrison et al., 1993), and CaM kinase II phosphorylation in the tail (Countaway et al., 1992), though the physiologic relevance of MAP kinase and CaM kinase II phosphorylation is unproven at present. Dephosphorylation by protein-tyrosine phosphatases, including SHP-1 and SHP-2 which are activated by EGFR, also contributes to limiting EGFR signaling (Stein-Gerlach et al., 1998). Of special interest for prostate biology is that the prostatic acid phosphatase preferentially targets EGFR, shutting it off (Lin and Clinton, 1988). The importance of these negative regulatory mechanisms was shown by the hyper-responsiveness of cells expressing genetically engineered EGFR (Wells et al., 1990, 1991); cells expressing a kinase-active receptor which fails to undergo ligand-induced degradation became transformed and formed tumors in murine hosts.

The biologic roles of the numerous intracellular pathways activated by EGFR signaling are only now being assigned (for reviews see (Wells et al., 1998)). One pathway, via PLC γ activation, promotes cell motility by inducing cytoskeletal reorganization and lamellipod protrusion (Chen et al., 1996a; Segall et al., 1996; Ware et al., 1998). A second pathway, translocation of Grb2 and SHC leading to ras signaling and MAP kinase activation, is required for both mitogenesis and induced motility (Xie et al., 1998). Both of these pathways are operational in prostate cells (Turner et al., 1997; Xie et al., 1998). Obviously, both induced cell motility and cell proliferation are critical properties for organ development or carcinogenesis and progression (see below). It has been speculated that EGFR signaling may also be important for prostate functioning or maintenance of the epithelial lining of the urogenital tract, though these contentions remain unproven and the biochemical links speculative.

Role of EGFR in organogenesis

The first physiological role assigned to EGF was to promote epidermal development; newborn mice injected with this novel peptide demonstrated precocious eye opening and tooth eruption. Subsequent investigations demonstrated that systemic EGF was required for full placental and mammary gland development (Snedeker et al., 1991). However, only recently have investigators been able to parse out EGFR contributions to organogenesis by molecular engineering. Part of the difficulty in deciphering EGF functions is the redundancy of ligands; this has necessitated targeting the common receptor. Genetic deletion of the EGFR is lethal in late development or shortly after birth due to multi-organ

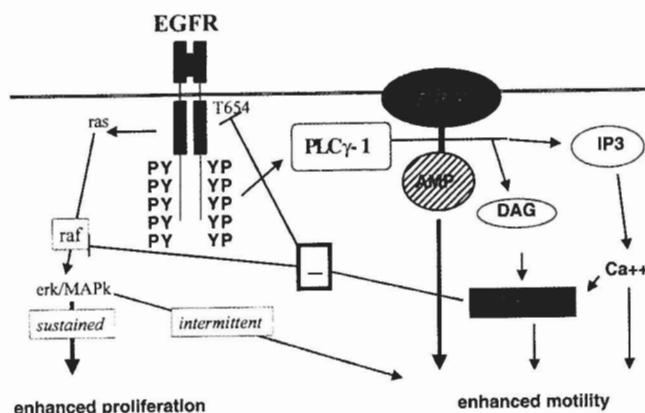


Fig. 2. Attenuation of EGFR signaling. The three best documented mechanisms by which EGFR signaling is limited involve (1) ligand-induced internalization and degradation, (2) feedback attenuation secondary to PKC-mediated phosphorylation at threonine 654, and (3) dephosphorylation of tyrosines (PY) by PTPases, including prostatic acid phosphatase (PACP) to restore the auto-inhibitory role of the EGFR carboxy terminal tail (Wells et al., 1998).

maldevelopment (Miettinen et al., 1995; Sibia and Wagner, 1995; Threadgill et al., 1995).

To overcome this lethal, multi-organ phenotype, in which some of the deficits are likely due to secondary events, other investigators have used overexpression of a kinase-inactive EGFR that provides a dominant-negative molecule in specific tissues (Amaya et al., 1991; Kashles et al., 1991; Redemann et al., 1992; Werner et al., 1993; Millauer et al., 1994). This approach has produced evidence which shows that EGFR plays a crucial role in the development of many organs. The targeted expression of dominant negative EGFR mutants in the basal layer of epidermis and outer root sheath of hair follicles using the promoter of the keratin 5 gene results in short and waved pelage hair and curly whiskers (Murillas et al., 1995). It has also been demonstrated that transgenic mice expressing a dominant negative mutant of mouse EGFR in their mammary gland showed a significant deficit in ductal branching and outgrowth and an overall decrease in the size of the mammary glands (Xie et al., 1997). However, as these dominant-negative EGFR constructs block all EGFR responses, the critical cell response induced by EGFR signaling is unknown.

We generated transgenic mice that express a dominant-negative form of PLC γ (PLCz) to investigate the role of EGFR-mediated motility in prostate and mammary gland development (Kim et al., 1998). The abrogation of PLC γ signaling had to occur postnatally and in tissue nonessential for life as this molecule is required for embryonic survival (Ji et al., 1998). We used the reverse tetracycline system to achieve expression of PLCz only in the presence of doxycycline. To target this exclusively to breast and prostate epithelial cells, the C3(1) prostatein promoter was utilized (Claessens et al., 1989). Prostatein, also known as prostate binding protein, is the major secretory protein of the rat ventral prostate. The C3(1) promoter limited expression of the reverse tetracycline repressor-HSV (herpes simplex virus) VP16 transcriptional activator fusion protein to the epithelial cells of the breast and prostate glands. We first investigated the role of the motility-associated PLC γ signaling pathway in the organogenesis of mammary and prostate glands by determining whether overexpression of dominant negative PLC γ is able to inhibit the normal development of these organs. The level of transgene expression was much higher than that of endogenous PLC γ , and the expression was restricted to the prostate and mammary glands. We observed developmental retardation in prostate development. The PLCz-expressing mice presented much less well-differentiated prostate and mammary glands than wild-type mice. The few lobules that developed during the first six weeks of life were significantly less folded. The retarded development is conditional, as withdrawal of doxycycline allowed for continued development of the prostate. The resultant picture in the mammary gland mirrored that obtained by Xie and colleagues (Xie et al., 1997). These results strongly suggest that early prostate and breast

development depends on EGFR signaling not of mitogenesis but of motility of the epithelial cells.

EGFR signaling in carcinogenesis

Understanding the control of epithelial cell growth, differentiation, and metabolism is crucial to understanding why many of these functions are controlled differently in transformed cancer cells. For example, transformed cells, unlike their normal counterpart, are often immortal, anchorage independent, and less apt to perform differentiated functions and have higher levels of expression for a wide variety of metabolic enzymes. Non-transformed epithelial cells maintain a tight balance between the effects of stimulatory and inhibitory factors. It has been proposed that transformed cells escape this control and require fewer exogenous factors than their normal counterparts (Holley, 1975). In fact, cancer cells very frequently require less serum to grow in culture than non-transformed cells. These observations formed the basis of the autocrine growth control hypothesis which states that transformed cells produce and respond to their own growth factors (de Larco and Todaro, 1978; Sporn and Todaro, 1980). This scenario has proved prescient in many carcinomas and has relevance to prostate carcinogenesis, in particular in regards to EGFR signaling.

An autocrine stimulatory loop of EGFR and TGF- α is a characteristic of all prostate tumor cell lines, PC-3, LNCaP, and DU-145 derived from metastatic lesions (Wilding et al., 1989; Connolly and Rose, 1990; MacDonald et al., 1990; Hofer et al., 1991). Immunohistochemistry for TGF- α shows negligible levels in normal epithelial cells, low levels of TGF- α in the epithelium of BPH and increased intensity of staining in prostate cancer cells (Harper et al., 1993). The least differentiated tumors show the most immunoreactive TGF- α . This reflects a change in ligand production, as the normal epithelial cells primarily produce EGF (Ching et al., 1993). However, this change is expected as the pH-dependent binding characteristics of TGF- α

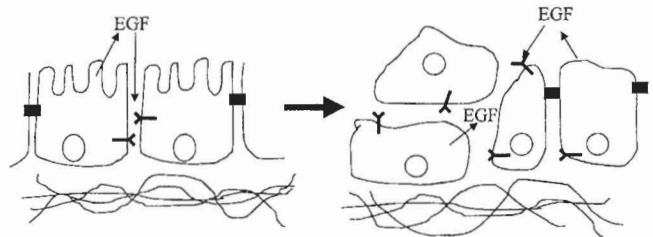


Fig. 3. Dysregulated EGFR signaling in early tumorigenesis. (Left) Early tumorigenic events begin to destabilize cell tight junctions, allowing access of the apically produced EGF to the EGFR on the basolateral aspect of the cell. (Right) Loss of tight junctions enables EGF to access and stimulate basolateral EGFR. Stimulated EGFR could significantly intensify the initial effects of tumorigenesis by further decreasing cell-cell contacts, reducing polarity, enhancing TGF- α expression through a positive feedback loop, and promoting dysplastic proliferation (multilayered epithelium) secondary to loss of contact inhibition.

EGF receptor signaling in the prostate

favor it as a stronger autocrine factor than EGF (Reddy et al., 1996). Interestingly, one might imagine a scenario that as the epithelial cells become disorganized and lose their tight junctions and strong polarity early in the transformation process, the apically-produced EGF gains access to the basolateral EGFR. As TGF- α expression is enhanced by EGFR signaling in a positive feedback loop (Bjorge and Kudlow, 1987) and EGFR signaling decreases cell-cell contacts and reduces polarity, this could represent a very early epigenetic event that further promotes prostate epithelial dysplasia and autonomous growth (Fig. 3).

Due to the nature of ligand-induced receptor downregulation, there have been conflicting reports as to the presence of EGFR in tumor specimens and cells. However, using message as an indirect indicator of EGFR levels, the level of EGFR mRNA has been found to be the highest in the androgen-independent human prostate cancer cell lines PC-3 and DU-145 (Morris and Dodd, 1990). However, these cells do not proliferate significantly when treated with EGFR ligands (Hofer et al., 1991; MacDonald and Habib, 1992). Instead, these cell lines produce TGF- α (MacDonald et al., 1990; Hofer et al., 1991), and at least DU-145 cells are dependent on this autocrine loop for cell proliferation (Turner et al., 1996). The decrease in cellular prostatic acid phosphatase, which preferentially dephosphorylates and inactivates EGFR, in prostate tumor cells may further contribute to enhanced EGFR signaling (Lin and Clinton, 1988). Therefore, the autocrine production of ligands may allow for the loss of steroid dependence (MacDonald and Habib, 1992). This, at least partial independence from the stromally-derived factors may enable the cells to be more distant from the stromal compartment, resulting in multilayered epithelium, and even grow in ectopic sites. Thus, the mitogenic response to EGFR signaling may come to play in prostate carcinogenesis rather than prostate development.

In order to test the function of EGFR signaling and this autocrine loop in prostate cancer, we engineered DU-145 cells to express various EGFR constructs. DU-145 overexpressing full-length EGFR were more invasive both in vivo and in vitro, and this was dependent on EGFR signaling (Xie et al., 1995; Turner et al., 1996). However, DU-145 cells overexpressing a dominant signaling-restricted EGFR, one that is fully mitogenic, were basically noninvasive. This suggested that the role of EGFR signaling at this stage of tumor progression did not involve mitogenesis. This finding was not unexpected as the correlation of increased EGFR signaling is with invasion and metastasis and not carcinogenesis in a wide variety of tumors, including glioblastoma multiforme and carcinomas of the stomach, bladder and breast (Libermann et al., 1985; Neal et al., 1985; Yasui et al., 1988; Klijn et al., 1992). Based on these findings, we had postulated that the role of EGFR signaling in prostate tumor invasion was to promote cell motility. This was tested by targeting a motility-specific signaling pathway, that involving PLC γ (Chen et al.,

1996b), by pharmacological or molecular inhibitors. Using either system of inhibition of PLC γ , tumor invasion was severely abrogated (Turner et al., 1996, 1997). Tumor growth in vivo and cell growth rate in vitro were unaffected. To determine whether this was unique to the DU-145 cell system, we have found that in vitro invasiveness of other prostate carcinoma lines and lines from breast and bladder carcinomas also requires an EGFR-PLC γ signaling pathway (Kassis et al., 1999). Thus, EGFR signaling promotes tumor invasion by enhancing cell motility. This would explain, at least partially, why prostate and breast tumors which show upregulated levels of EGFR have poorer prognoses (Surya and Provet, 1989; Klijn et al., 1992).

This still leaves open the question of whether EGFR-mediated mitogenesis contributes to prostate carcinogenesis. One possible function was discussed above, in which an early autocrine loop may contribute to dysplasia. A second event that may be dependent on EGFR mitogenesis is the growth of ectopic foci – metastases. In one experimentally-validated global concept of metastatic spread, the 'seed and soil' hypothesis, tumors metastasize preferentially to specific organs based upon the local environment, in particular matrix and paracrine factors. Prostate carcinomas display a strong predilection for a hierarchical metastatic spread both in de novo human tumors and animal models. This involves, after localized invasion, first regional lymph nodes and axial bone marrow, and only later are other organs involved. Experiments have suggested that a diffusible factor produced by the stromal cells of these organs (a paracrine growth factor) supports prostate tumor cell growth. However, these local stromal cells do not fully promote normal prostate epithelial growth and function. We, and others, have proposed that the EGFR autocrine signaling provides a vital growth signal that allows for the cells to require in addition only a subset of normal prostate gland compliment of growth and survival factors to be supplied from the host environment; by analogy to the 'seed and soil', that EGFR autocrine stimulation is the equivalent of partial self-fertilization. In support of this hypothesis, prostate carcinoma lines require the EGFR autocrine signaling for cell growth. Inhibition of EGFR signaling cannot be bypassed by activation of the receptors for insulin, IGF-I, bFGF or PDGF. This

Table 2. Changing roles of EGFR signaling during prostate development and carcinogenesis.

STAGE	ROLE	CELLULAR RESPONSE
Prostate Development	Branching and folding	Motility (PLC γ -dependent)
Mature Prostate	Functioning Repair	Unknown Mitogenesis
Carcinogenesis	Dysplasia Invasion Metastasis growth	Loss of tight junctions and polarity Motility (PLC γ -dependent) Mitogenesis (autocrine signaling)

hypothesis can only be validated by allowing for tumor dissemination and then eliminating EGFR-mediated mitogenic signaling.

In short, studies show multiple roles for EGFR signaling which contribute to, or stem from the changing nature of the prostate epithelium during carcinogenesis and tumor progression (Table 2). At an early stage, EGFR activation by stromally-derived TGF- α and leakage from prostatic fluid EGF may contribute to hyperproliferation and allow a multi-layered epithelium and dysplasia. During the clinical critical invasive transition, EGFR-mediated motility and not mitogenesis comes to the fore. However, for the disseminated tumors to grow and form clinically-relevant metastases, autocrine growth stimulation via EGFR may be crucial.

Conclusion

This limited review has highlighted, almost exclusively, the role of the EGFR signaling in prostate epithelial development and tumorigenesis. This does not imply that other factors are unimportant; other investigations have demonstrated critical roles for growth factors, matrix components and genetic changes in both prostate development and function and carcinogenic transformation. Rather we have focused on the EGFR system as it is the best characterized in the prostate and current data support a central role for this receptor.

Understanding the physiological and pathological roles of EGFR may yield not only insight into a fascinating biological event, prostate development and function, but also novel therapeutic targets for a clinically devastating disease, prostate cancer. Prostate cancer has confounded treatment due to its slow growth which renders the heretofore anti-mitotic approaches useless and dangerous and its escape from the androgen dependence of a epithelial-stromal paracrine system cause hormonal treatment to be only palliative and short-lived. EGFR signaling could be a novel target to limit prostate cancer spread and growth. At the least, EGFR-mediated motility is required for invasiveness, a tumorigenic event which has not yet been successfully attacked, and this can be abrogated either by inhibiting EGFR or PLC γ signaling. Inhibiting signaling of this latter target, PLC γ , may have fewer untoward side effects, as other functions of the ubiquitous EGFR would be only marginally effected. In addition, EGFR signaling may promote both early tumorigenesis and, more important clinically, metastatic growth. There is great optimism that a deeper appreciation of EGFR signaling in the prostate and prostate cancer will lead to novel, and efficacious treatment for prostate cancer.

Acknowledgements. We thank the members of the Wells lab and Turner lab for helpful discussions and criticisms. The work discussed herein and this review were supported by grants from the Veterans Administration and the National Institutes of Health (NIGMS and NCI).

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Accepted February 19, 1999