MINIREVIEW

The Role of CSF-1 in Normal and Neoplastic Breast Physiology (44337)

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Abstract. **Colony stimulating factor (CSF-1) and its receptor (CSF-1R, product of c-*fms* proto-oncogene) were initially implicated as essential for normal monocyte development as well as for trophoblastic implantation. However, recent findings have suggested that CSF-1 and CSF-1R might have additional roles in mammary gland development during pregnancy and lactation. Studies with osteopetrotic (*op*$^-$/*op*$^-$) mice, which bear a specific mutation that inactivates the CSF-1 gene, demonstrated that *op*$^-$/*op*$^-$ mothers are incapable of normal milk production due to the incomplete development of their mammary glands during pregnancy. Also, significant increases in the levels of CSF-1 and CSF-1R proteins are observed in the epithelial cells of mammary gland during pregnancy and lactation. *In vitro* studies investigating the effect of the three major lactogenic hormones (prolactin, insulin, and glucocorticoids) on the expression of CSF-1 and CSF-1R have demonstrated that expression of CSF-1 can be regulated by prolactin and insulin whereas CSF-1R expression is regulated by glucocorticoids. This apparent role for CSF-1/CSF-1R in normal mammary gland development is very intriguing because this receptor/ligand pair has also been found to be important in the biology of breast cancer, where they regulate tumor cell invasion by a urokinase-dependent mechanism. This review aims to summarize recent findings on the role of CSF-1 and its receptor in normal and neoplastic mammary development which may elucidate potential relationships of growth factor–induced biological changes in the breast during pregnancy and tumor progression.**

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Biology and Action of CSF-1 and its Receptor

Macrophage colony stimulating factor (CSF-1) was first identified as a haematopoietic growth factor that stimulates the proliferation, differentiation, and survival of monocytes, macrophages, and their bone-marrow progenitors (1–3). CSF-1 is an extensively glycosylated proteoglycan synthesized in both soluble and membrane-bound forms (3). All known forms of CSF-1 bind to a unique cell surface receptor encoded by the cellular homolog of the retroviral oncogene v-*fms,* the c-*fms* proto-oncogene (4). The CSF-1 receptor (CSF-1R) is a member of a family of tyrosine kinase receptors, which includes the α and β forms of platelet-derived growth factor, the receptor of Steel factor (the product of c-*kit* proto-oncogene), and the receptor for basic fibroblast growth factor (5–6). Activation of CSF-1R by its ligand triggers a series of rapid events including receptor dimerization, phosphorylation on at least six intracellular tyrosine residues, and association of the intracellular domain of the receptor with a variety of cytoplasmic effector proteins that activate multiple signal transduction pathways controlling cell proliferation and differentiation (7–8). For

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example, several typical macrophage traits such as chemotaxis, phagocytosis, the synthesis and secretion of proteolytic enzymes (e.g., urokinase-type plasminogen activator, thromboplastin), and other cytokines (e.g., interleukin-1, TNFα, G-CSF, and interferon) are induced by CSF-1 (9–10).

Several years ago, additional roles for CSF-1 in nonhaematopoietic systems were suggested by detection of high level synthesis of CSF-1 by the pregnant uterus coupled with high level expression of its receptor in the placental trophoblast (11–12). Several independent studies have demonstrated that activation of trophoblastic CSF-1R by the locally high levels of CSF-1 produced by the endometrial epithelium is essential for normal embryonic implantation and placental development (13–14).

Furthermore, several recent findings have implicated a role for CSF-1 and its receptor in the mammary gland during pregnancy (15–16). In lactating breast epithelium, very high levels of CSF-1 and CSF-1R antigens are expressed (16) as opposed to the undetectable levels of CSF-1R and low levels of CSF-1 found in resting ductal epithelium (17). Such potential roles for CSF-1 and CSF-1R in mammary gland development are intriguing given their recently demonstrated biological actions and clinical significance in human breast carcinoma (17). In this review we focus on recent attempts to understand the biological significance of the expression of the CSF-1 and its receptor during normal and neoplastic mammary gland development.

Physiology of the Mammary Gland during Pregnancy and Lactation

The influence of sex steroid hormones, peptide hormones, and growth factors induces breast tissue to progress through a series of distinct developmental stages during fetal life, infancy, childhood, puberty, pregnancy, and menopause (18–19). Each developmental stage is the end result of complex, stage-specific coordinated sequences of cell proliferation, migration, tissue remodeling, and differentiation of mammary epithelial and stromal elements. For example, the lactating mammary gland exhibits highly differentiated epithelial and stromal structures distinct from those seen at any other developmental stage (19–20).

The first stage of lactogenic development is observed during early pregnancy and is characterized by the proliferation and growth of the cells of the distal portion of the ductal tree to form alveolar structures in response to elevated levels of estrogen and progesterone (20). As pregnancy proceeds, these alveolar lobules increase in size and number with new lobules developing from the lateral walls and termini of ducts. Concomitantly, stromal fat decreases, and the developing glandular structures become highly vascularized. Alveolar epithelial cells are cuboidal and contain large cytoplasmic lipid vesicles. This enhanced proliferation of lobuloalveolar structures requires estrogen, progesterone, prolactin, adrenal corticosteroid, insulin, and growth factors (20). From midpregnancy onward, the rate of mammary epithelial proliferation begins to decline, and the alveolar epithelium differentiates to assume a presecretory function (20). After parturition, sudden decreases in the levels of circulating estrogens and progesterone and increases in levels of the lactogenic hormones (prolactin, glucocorticoids, and insulin) signal the onset of lactation (21). The alteration of hormonal milieu further stimulates the differentiation of the mammary gland to extrude secretory vacuoles that contain fats, a variety of milk proteins such as casein, α-lactalbumin, transferrin/lactoferrin, and many growth factors and other proteins whose functions remain unclear (e.g., whey acidic protein = WAP).

CSF-1 in Human Milk

Human milk has been shown to contain large amounts of biologically active CSF-1 (22). Milk CSF-1 is an 80-kD disulfide-linked homodimer similar to that of serum or urine CSF-1. Maximal milk CSF-1 concentrations can be observed on Day 2 postpartum with an average of 28,000 U/ml; however, one month after delivery, milk CSF-1 concentration has rapidly declined to less than 10,000 U/ml (22). The source of milk CSF-1 was investigated in lactating mammary glands by immunohistochemical staining and *in situ* hybridization and found to be expressed locally by the secretory epithelial cells of the mammary gland (16, 22). One report suggested that elevated concentrations of milk CSF-1 during lactation are the mere result of increased numbers of milk secreting epithelial cells, since normal nonlactating epithelial cells synthesize significant amounts of CSF-1 (22). However, our investigations summarized below clearly demonstrate that the levels of CSF-1 mRNA and protein are significantly upregulated in lactating epithelial cells *in vivo* and *in vitro* (16).

Expression Pattern of CSF-1 and CSF-1R in Mammary Gland during Pregnancy and Lactation

Several immunohistochemical (IHC) and *in situ* studies have revealed that normal resting, nonlactating, human breast tissue expresses a low but significant level of CSF-1 without expressing its receptor (17, 23). Such resting state CSF-1 expression has been localized to cells of both the mammary epithelium and the stroma. Levels of expression of CSF-1 and CSF-1R during lactation have also been studied immunohistochemically in paraffin sections from prelactating and lactating breast biopsies (16). All biopsies obtained from actively lactating women and half of the biopsies taken from women in the last trimester of pregnancy showed significant expression of CSF-1 and CSF-1R antigens in the epithelial cells of ducts and alveoli. The intensity of the observed IHC staining is comparable to that observed when either breast carcinomas or syncytiotrophoblasts are stained for CSF-1R antigen and when breast carcinomas are stained for CSF-1 (23). Similar increases were observed in the levels of CSF-1 and its receptor in mouse mammary gland development during pregnancy. Both antigens were

found to be highly elevated in the lactating epithelial cells of the postpartum murine mammary gland (unpublished data).

Effects of Sex Steroid Hormones on the Expression of CSF-1 and CSF-1R during Pregnancy

The first real evidence for hormonal responsiveness of CSF-1 expression was derived from *in vivo* studies of the role of CSF-1 in mouse female reproductive organs, placenta and uterus (24). These studies revealed that CSF-1 levels could be increased by estrogen up to 1000-fold in the mouse uterus during pregnancy. In ovariectomized mice, concomitant administration of estradiol 17β and progesterone resulted in a dramatic increase in CSF-1 mRNA expression to levels comparable to those detected during pregnancy, suggesting that progesterone is also required for the observed upregulation of CSF-1 (11). In human pregnancy, CSF-1 concentration was also found to be elevated in the first trimester endometria over concentrations found in the nonpregnant uterus (25). CSF-1 was also detected in mouse and human placenta at consistently high levels (24–25).

CSF-1R expression shows distinctive patterns during mouse and human pregnancy with CSF-1R mRNA first detected in mouse oocytes prior to fertilization. In fertilized oocytes, CSF-1R expression could be observed from the 2–4-cell stage until implantation (26). After implantation, CSF-1R mRNA was detectable in the decidua (26). In human pregnancy CSF-1R expression has been observed in both placenta and uterus (27). In placenta the most intense expression was observed in syncytio- and invasive trophoblast, which contain proliferating, invasive cells. To date, the mechanisms that underlie the above-described hormonal regulation of CSF-1R expression in placenta and uterus remain unknown; however, it has been suggested that estradiol 17β and/or progesterone might regulate CSF-1R synthesis to synchronize the maternal preparation of the uterus with embryonic development (13). Analysis of CSF-1R promoter sequence suggested the presence of both estrogen and progesterone receptor response elements (unpublished data) in addition to glucocorticoid response elements (GREs) discussed below.

Effects of Lactogenic Hormones on the Expression of CSF-1 and CSF-1R in Mammary Epithelial Cells

The dramatic increases in the expression of CSF-1 and CSF-1R antigens in lactating mammary epithelial cells strongly suggest that lactogenic hormones (prolactin, insulin, and glucocorticoids) might regulate the expression of both genes. The first evidence for such regulation stems from our own studies of the hormonal responsiveness of CSF-1R expression in neoplastic mammary epithelial cell lines (28). Levels of CSF-1R (c-*fms*) transcripts rapidly increase after dexamethasone (a potent synthetic glucocorticoid) treatment from barely detectable levels in untreated cells to levels 6-fold higher by 2 hr and 25–50-fold higher by 8–24 hr. A similar but much weaker effect was observed with progestins (most likely by cross-reactivity of high levels of progestins at the glucocorticoid receptor), but no effects were observed in these cell lines with prolactin, insulin, estrogens, androgens, and mineralocorticoids (23). Glucocorticoid-induced increases in the level of c-*fms* mRNA and protein could be blocked by pretreatment of breast carcinoma cells with the potent glucocorticoid competitive antagonist, mifepristone (RU486), a result that demonstrates that glucocorticoid-induced changes in c-*fms* transcript levels are mediated through a ligand-activated glucocorticoid-induced receptor (28). Employing promoter/receptor constructs in which each of the known c-*fms* promoters was cloned upstream of firefly luciferase reporter gene, we demonstrated that most of the observed effects of glucocorticoids on c-*fms* transcript levels in mammary epithelial cells resulted from hormone stimulation of c-*fms* gene transcription (28). Computer analysis of the sequence of the *fms* promoters revealed that both contained several potential "glucocorticoid response elements" (GREs). In each case, elimination of the GRE closest to the promoter (by *in situ* point mutagenesis or deletion) abolished all glucocorticoid stimulation of promoter activity in reporter gene constructs, demonstrating that the observed GRE elements found in the c-*fms* promoters are functional (28). Taken together, these results make glucocorticoids the only known physiologic or pharmacologic agents that, to date, have been shown to be able to significantly increase the rate of c-*fms* transcription in any cell type or experimental system.

The potential regulation of the expression of CSF-1 and c-*fms* gens by lactogenic hormones was studied *in vitro* on organ cultures of normal human breast exposed to lactogenic hormones: insulin, prolactin, and glucocorticoids. The organ cultures were prepared from slices of reduction mammoplasty biopsies obtained from normal-cycling females (16). Mammary epithelial organ cultures were maintained in defined media supplemented with certain cytokines and the three essential lactogenic hormones (prolactin, glucocorticoids, and insulin) for 14 days. CSF-1 and c-*fms* expression were monitored by IHC studies on the paraffin sections of the organ culture samples. In agreement with previous *in vivo* IHC results on lactating breast specimens, significant increases in the of CSF-1 and c-*fms* genes were observed in the epithelial cells of mammary gland organ samples exposed to lactogenic hormones *in vitro* (16). The increase in CSF-1R, but not in CSF-1, expression was shown to be blocked by mifepristone (RU486), a glucocorticoid competitive antagonist, consistent with the effects of this agent on breast carcinoma cell lines (28). The latter result strongly suggests that glucocorticoids can regulate the expression of c-*fms* (CSF-1R), without affecting the expression of its ligand during lactogenic differentiation. This finding also indicates that insulin and prolactin might be essential for upregulation of CSF-1 expression during lactogenesis.

The role of prolactin and insulin was demonstrated by *in vitro* studies of CSF-1 expression in the normal mam-

mary epithelial cell line (HC11) cultured in the presence of lactogenic hormones. The HC11 cell line was originally isolated from midpregnant mouse mammary gland tissue (29) and has been found to express significant levels of CSF-1 without expressing its receptor (30). HC11 cells retain important features of normal mammary epithelial cells as evidenced by their ability to differentiate and synthesize the milk protein β-casein after exposure to lactogenic hormones (29). These features make HC11 cells an ideal model for the study of gene expression during lactogenesis. Northern blot analysis demonstrated that exposure of HC11 cells to insulin or prolactin for 24 hr, could increase CSF-1 mRNA levels by 5- and 10-fold, respectively (16). When HC11 cells were treated with insulin and prolactin together for the same time period, a significantly greater effect (~30-fold) was found on the induction of CSF-1 mRNA levels (16). Glucocorticoids had no effect on CSF-1 mRNA levels on their own, nor did they potentiate the effects of either of the two peptide hormones, insulin and prolactin, over those seen with each hormone alone (16).

Regulation of CSF-1 expression in many cell types appears to be a consequence of alteration of both transcript half-life and transcription rate (31–32). Our own preliminary studies revealed that increases in CSF-1 mRNA levels by the peptide hormones prolactin and insulin are the consequence of both upregulation of the promoter activity of CSF-1 and prolonation of CSF-1 mRNA half-life by those lactogens (unpublished data). These findings are fascinating since they appear to add CSF-1 to the growing list of so-called "milk proteins" whose expression is regulated by lactogenic hormones. Such an effect not only helps to account for the high levels of CSF-1 expression observed in lactating mammary epithelial cells *in vivo* and *in vitro* but also for the presence of significant levels of CSF-1 in milk. They also strongly suggest that the concerted action of the three lactogenic hormones (glucocorticoids, insulin, and prolactin) induces the expression of both CSF-1 and its receptor in mammary epithelial cells, providing an autocrine loop for this growth factor/receptor pair during pregnancy and lactation.

The Role of Expression of CSF-1 and CSF-1R in Mammary Gland Development during Pregnancy

To date only one *in vivo* study has investigated the potential role of CSF-1/CSF-1R during mammary gland development (15). Homozygous mutant (op^-/op^-) mice, whose specific genetic defect inactivated both copies of the CSF-1 gene, are osteopetrotic consequent to a deficiency in osteoclast function, have severely reduced numbers of macrophages, and have reduced fertility and scant milk production after parturition. Investigation of the mammary gland development in op^-/op^- mothers revealed that the observed lactational defect was the result of incomplete mammary gland ductal growth, precocious development of the lobuloalveolar system, and inability to secrete milk proteins during lactation. These findings clearly demonstrated that CSF-1 regulates multiple stages of mammary gland differentiation from ductal and alveolar development to milk protein secretion and strongly suggest that CSF-1 is a necessary factor for normal mammary gland development during pregnancy and lactation.

Expression of CSF-1 and CSF-1R in Breast and Other Epithelial Carcinomas

As described above, CSF-1R was originally identified as the oncogene (v-*fms*) transduced by a feline oncogenic retrovirus, which causes fibrosarcomas in cats (4). In earlier studies investigators questioned whether abnormal expression of the cellular counterpart (c-*fms*) or mutation of c-*fms* gene can render normal human cells tumorigenic. Multiple studies have investigated the expression pattern of CSF-1 and CSF-1R in normal and neoplastic tissues and cell lines (17, 23, 33–41). Abnormal expression of CSF-1R—with or without CSF-1—has been documented in a wide variety of human carcinomas and carcinoma-derived cell lines including tumors of epithelial origin such as carcinomas of breast, ovary, endometrium, lung, kidney, and pancreas (17, 23, 33–41).

Previous IHC and *in situ* studies have demonstrated that 58% of all and 85% of invasive breast carcinomas expressed CSF-1R at levels comparable to those observed in trophoblast and macrophages and, in these cases, CSF-1R expression was clearly localized in the neoplastic epithelial cells of the tumors as well as stromal macrophages (23, 38). Furthermore, several earlier studies reported that expression of CSF-1R in ovarian and endometrial carcinomas strongly correlated with such adverse prognostic features as high histologic grade and advanced clinical stage at presentation (40–41). The prognostic value of CSF-1R expression in mammary epithelial tumors has been documented recently in a study of ipsilateral breast cancer recurrence (42). The activation state of CSF-1R was also studied on breast carcinoma specimens by IHC studies using an antibody that recognized CSF-1R only after activation by its ligand or activating mutation(s). This study revealed that 52% of CSF-1R positive breast carcinoma expressed activated CSF-1Rs (43).

Activation of CSF-1R by its ligand could occur in invasive tumor cells where CSF-1R and CSF-1 are coexpressed (i.e., autocrine activation), or tumor cell CSF-1Rs could also be stimulated indirectly by CSF-1 synthesized by infiltrating monocytes or fibroblasts found in the tumor stroma (i.e., paracrine activation). Recent findings suggested that the effect of autocrine activation of CSF-1 receptor might predominate over paracrine effects, at least in ovarian carcinoma (44).

Given that tumor cells synthesize CSF-1 in quantities high enough to be readily detected by IHC studies, it is not surprising that significant levels of apparently tumor-produced CSF-1 are found in the serum and ascites of patients with breast and other carcinomas (45–51). High circulating levels of CSF-1 have been reported to correlate

strongly with active or recurrent disease in patients with ovarian and endometrial carcinoma (48–51), and with rapidly progressive metastatic disease in patients with breast carcinoma (45–47). Serum levels of CSF-1 in terminally ill breast cancer patients were reported to be increased to more than 10-fold normal levels whereas IHC evidence of CSF-1 expression was found to be higher at the site of metastatic recurrence (45–47).

Also, individual, stromally invasive breast carcinoma cells consistently expressed CSF-1, which was often not expressed in adjacent noninvasive, *in situ* carcinoma (46–47). In addition, expression of CSF-1 by breast carcinoma has itself been associated with genomic amplification of *int*-2 and *erb* B-2/neu proteins whose amplification and/or overexpression were found to be indicators of poor prognosis in breast carcinoma (45, 52). CSF-1 expression in primary breast adenocarcinomas also correlated with infiltration of inflammatory cells and prognosis (53). This latter result strongly indicates that tumor-produced CSF-1 can recruit and activate large numbers of monocytes which in turn release trophic cytokines and other growth factors to enhance tumor cell growth. This hypothesis is also supported by a recent finding in which exogenous CSF-1 enabled macrophages to stimulate tumor growth in the CSF-1-deficient mouse (op^-/op^- mouse, see above) model system (54).

The above-mentioned *in vivo* findings are very intriguing, but unfortunately they do not explain the exact role of CSF-1 and its receptor in epithelial tumor development. To characterize such a role, we also need *in vitro* systems (e.g., established epithelial cell lines) in which we can study the effect of the abnormal expression of CSF-1R with or without the expression of CSF-1.

Transformation Potential of c*fms* Proto-Oncogene

A feline sarcoma virus containing the v-*fms* sequence was shown to induce fibrosarcomas when inoculated into cats (55). Analysis of the v-*fms* gene product demonstrated that it is constitutively active as a tyrosine kinase and provides tonic signals for cell growth in the absence of CSF-1 (4, 6). The sequence changes necessary to convert the c-*fms* gene to an active oncogene were analyzed by comparison of v-*fms* and the feline cellular homolog c-*fms* gene (56–60). Construction of chimeric *fms* proteins along with site-directed and random chemical mutagenesis demonstrated that multiple changes are necessary for the full activation of the oncogenic potential of the c-*fms* gene (58, 60). These include several mutations in the extracellular domain as well as truncations and point mutations in the carboxy terminus of the c-*fms* gene (56–60).

In addition, previous studies investigated a potential autocrine mechanism of transformation caused by overexpression and/or inappropriate expression of the normal c-*fms* gene in nonmalignant cells synthesizing endogenous CSF-1. These studies revealed that overexpression of normal c-*fms* gene (cloned into retroviral vector and transfected into CSF-1-expressing normal cells) can be sufficient to induce a fully transformed phenotype in fibroblast and epithelial cells (30, 61). This latter transformation mechanism provides an example of the importance of the abnormal (uncontrolled) coexpression of a growth factor/receptor pair in the initiation of a neoplastic phenotype in nonmalignant cells. However, we cannot rule out the possibility that both of the host cell lines used in these experiments might also contain uncharacterized mutations that could account for some of the aspects of their "transformed" phenotypes.

Expression of CSF-1 and CSF-1R by Neoplastic Mammary Epithelial Cell Lines

In vitro expression of CSF-1 transcript and protein have been observed in mammary epithelial cells of benign (e.g., murine HC11 cells) or neoplastic (e.g., human SKBR3 cells) origin whereas the expression of functional CSF-1R has been documented in several breast carcinoma cell lines (e.g., human BT20 and SKBR3 cells, Ref. 16–17, 23, 30). Sequence analysis of CSF-1R mRNAs from these neoplastic cell lines indicated that their protein coding sequences do not significantly differ from those expressed by normal macrophages or placental trophoblasts (23). Hence activation of CSF-1R in breast carcinoma is probably not due to any known "activating" mutation(s) previously described in the c-*fms* gene sequence. For that reason, several years ago we and other began to study the effect of the activation of CSF-1R by its ligand on several cellular characteristics of normal and neoplastic mammary epithelial cells.

CSF-1/CSF-1R Induced Cellular Invasiveness and Anchorage-Independent Growth in Mammary Epithelial Cells

Several reports have demonstrated that CSF-1R activation regulated normal cellular migration of macrophages through urokinase-type plasminogen activator-dependent (uPA) mechanisms (3, 62–63). The first indirect evidence for a similar involvement of CSF-1R in mammary epithelial cell invasion came from a study that investigated CSF-1R positive breast carcinoma cell line migration through amniotic membrane (64). It was shown that dexamethasone (which increases CSF-1R levels, see above) and CSF-1 together could significantly enhance *in vitro* invasion and uPA production of these carcinoma cells (64). Also, it was reported that uPA mediates stimulation of invasion by CSF-1 in ovarian carcinoma cells *in vitro* (65). In addition, transfection of the wild type c-*fms* gene into a normal, noninvasive mammary epithelial cell line (HC11), which expresses CSF-1, resulted in a dramatic stimulation of the invasive phenotype and anchorage-independent growth of these cells (30). The stimulatory effect of c-*fms* expression on invasion by HC11 cells was shown to be blocked efficiently by specific inhibitors of the uPA/collagenase proteolytic cascade (30). In the same study the importance of the phosphorylation of specific tyrosine residues of CSF-1R in the autocrine activation of the CSF-1 receptor was also studied. Two major autophosphorylation sites were mutated

(Tyr- > Phe 807 or Tyr- > Phe 721) and analyzed for their potential effect on CSF-1 receptor–induced invasion and anchorage-independent growth in HC11 cells. Previous analyses of such phosphorylation site mutants showed that Tyr807 and 721 play important roles in coupling the CSF-1 receptor to distinct intracellular signal transduction pathways (66–67). The Tyr807 site in particular was demonstrated to be crucial for CSF-1-dependent monocytic differentiation (66), whereas the Tyr721 site of the CSF-1 receptor is required for CSF-1–dependent mitogenesis in macrophages (67). While mutations at Tyr807 significantly reduced the stimulatory effect of c-*fms* expression on the invasive ability of HC11 cells *in vitro* and *in vivo,* mutation at Tyr721 of c-*fms* had no effect on *in vitro* invasion (30). In contrast, mutations of 721, but not of Tyr 807, had a significant effect on c-*fms*–induced anchorage-independent growth and the *in vivo* metastatic potential of the transfected cells (30). These data clearly demonstrated that anchorage-independent growth and cellular invasiveness, two crucial steps in tumor development, could be regulated independently by separate sites of tyrosine-phosphorylation of CSF-1R. Because phosphorylation of these specific tyrosines activates completely separate secondary intracellular pathways (7–8), these findings also indicated that anchorage-independent growth and invasiveness might be regulated independently by CSF-1R in mammary epithelial cells.

Potential Role of CSF-1/CSF-1R in Neoplastic Mammary Epithelial Cells

The parallels in the observed patterns of expression of CSF-1 and CSF-1R during lactogenic differentiation and tumor development become somewhat less surprising when one recognizes that many of the events that occur during normal mammary gland development have strong similarities to those observed during tumor progression. For example, normal resting ductal and alveolar epithelial elements proliferate and synthesize a variety of proteases (68) able to digest, remodel, and "invade" stromal elements to produce the extensively branched ductal and alveolar structures characteristic of the lactating breast. While in normal mammary development these processes are strictly controlled not only by hormones (20) but also by a variety of growth factors (69–71), adhesion molecules (72–73) and transcription factors (74–76), it is not difficult to imagine that abnormal activation of this otherwise normal developmental program could yield a malignant phenotype in mammary epithelial cells (77). Thus, even nonmalignant precursors of malignant cells possess the metabolic program to direct rapid proliferation and secretion of proteolytic enzymes employed by neoplastic cells to invade the stroma and eventually disseminate (78).

For example, in normal mammary epithelial cells, activation of the CSF-1/CSF-1R–induced signaling pathways dramatically stimulates cellular invasiveness through a uPA-dependent pathway *in vitro* (30). This same pathway might be very crucial to normal mammary development since it has been shown that during lactogenesis ductal and alveolar epithelial elements express CSF-1/CSF-1R and synthesize a variety of proteolytic enzymes including uPA and other gelatin and casein-degrading proteinases (68, 78). In breast cancer, CSF-1/CSF-1R and uPA expression each appear to correlate with tumor invasiveness and an adverse clinical prognosis (47, 79). Taken together these parallel observations strongly suggest that one of the major functions for CSF-1/CSF-1R in normal and neoplastic cells might be the activation of uPA-dependent proteolytic pathways and the induction of cellular invasiveness (46).

Summary

CSF-1 and CSF-1R appear to be important to the physiology of normal and neoplastic mammary glands. Levels of CSF-1 and CSF-1R expression are upregulated by hormones implicated in normal mammary gland development. Results from CSF-1–deficient mice suggest that this ligand/receptor pair is important in multiple stages of mammary gland differentiation from ductal and alveolar development to milk protein secretion. In breast cancer, levels of CSF-1 and CSF-1R expression have been shown to correlate with tumor cell invasiveness and adverse clinical prognosis. In summary, these findings clearly demonstrate that a greater understanding of the role of growth factors and their receptors in the normal mammary gland development has obvious relevance to the field of breast cancer, and it is quite possible that hormonal or pharmacologic therapies that interfere with the expression of CSF-1 and its receptor and/or the intracellular signal transduction pathways they regulate could be useful adjuncts to the treatment of human breast cancer.

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