LECTURE

Protein tyrosine phosphorylation signaling in the differentiation of human endometrial stromal cells

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(Received for publication on February 27, 2002)

Abstract. Reversible protein tyrosine phosphorylation, coordinately controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), is a critical element in signal transduction pathways regulating cell growth, differentiation, apoptosis, and tumorigenesis. The differentiation of human endometrial stromal cells (decidualization) is crucial for successful embryo implantation and maintenance of pregnancy; however, little is known about the molecular events involving tyrosine phosphorylation, PTKs, and PTPs in the process of decidualization. We have previously reported that the tyrosine kinase activity of c-Src belonging to the Src family kinase is increased together with altered tyrosine phosphorylation of several cellular proteins in the *in vitro* model of decidualization. Focal adhesion kinase (FAK) and paxillin are known to form a complex with c-Src at the focal contacts and to participate in the integrin-mediated signal transduction as c-Src substrates. Those focal adhesion proteins, however, are not hyperphosphorylated on tyrosine during decidualization. Moreover, the loss of focal adhesions and the disorganization of the actin-based cytoskeleton were observed in decidualized stromal cells, suggesting that the escape from regulation by c-Src may be in part due to the decidualization-induced disruption of the interaction between the focal adhesion proteins and c-Src. These findings collectively indicate that decidual c-Src may activate signaling pathway(s) different from the integrin-mediated signaling cascade involving FAK and paxillin. This review summarizes our recent studies on the tyrosine phosphorylation signaling pathway(s) in decidualization. (Keio J Med 51 (2): 93–99, June 2002)

Key words: tyrosine phosphorylation, c-Src, endometrium, decidualization, focal adhesion

Tyrosine Phosphorylation and Protein Tyrosine Kinases

Protein phosphorylation on serine, threonine, and tyrosine residues is deeply involved in intracellular signal transduction. In particular, reversible protein phosphorylation on tyrosine residues, controlled coordinately by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), plays a pivotal role in a wide variety of biological processes including cell growth, differentiation, apoptosis, and tumorigenesis.[1] The PTKs can be divided into two main categories: the receptor-type transmembrane PTKs (RPTKs) and the non-receptor PTKs (NRPTKs).[1] Most members of the former class function as receptors for growth- or differentiation factors, while the physiological function of the NRPTKs is far less understood.[1] The NRPTKs can currently be further subdivided into 10 families, based on structural similarities and sequence homology.[1] Among them, the Src family tyrosine kinases including c-Src and Fyn have been well characterized as key molecules controlling the dynamic process of tyrosine phosphorylation in various types of cells.[2] However, very little is known about NRPTK- and RPTK-mediated signaling(s) in a variety of human female reproductive processes.

Decidualization

Decidualization is the progesterone-induced differentiation of fibroblast-like stromal cells of the proliferative estrogen-primed endometrium into decidual cells,

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which are easily distinguishable histologically as the larger and rounder cells appearing around the spiral arteries and eventually spreading through the most part of the endometrium in the luteal phase of the menstrual cycle.[3] Following embryo implantation, decidualization persists and extends throughout the endometrium, leading to the formation of the pregnancy decidua. This morphological change is accompanied by the biochemical expression of a number of biological substances.[4] These decidualization-associated factors, in turn, act as local regulators of both decidual and trophoblast functions.[5,6] Thus, decidualization is crucial for successful embryo implantation and maintenance of pregnancy.

In the presence of estrogen and progesterone, endometrial stromal cells (ESC) isolated from human endometrium can exhibit morphological and functional changes *in vitro* that mimic *in vivo* decidual transformation.[7,8] With the development of those *in vitro* models of decidualization of ESC, many studies have addressed the molecular mechanisms underlying decidualization.[4] Intracellular signaling molecules and pathways responsible for decidualization, however, have not been fully elucidated. Increasing bodies of evidence have implicated the activation of cAMP/protein kinase A-mediated signaling cascade(s) in decidualization,[9–11] which alone cannot substantially account for the multiple aspects of functional and morphological decidualization.

Alterations in the Profile of Phosphotyrosinyl Proteins and Kinase Activation of c-Src Tyrosine Kinase Concomitant with its Decreased Dephosphorylation upon *In Vitro* Decidualization of ESC

To explore possible decidual PTK-mediated signaling pathway(s), we first examined the profile of phosphotyrosinyl proteins in *in vitro* decidualized ESC using immunoblotting with the anti-phosphotyrosine (anti-PY) antibody 4G10. As shown in Fig. 1A, there were differences in the intensities of several phosphotyrosinyl proteins between the untreated and 17-β estradiol (E_2) + progesterone (P_4)-treated ESC (upper panel).[12] A thick band of ~60 kDa was clearly observed in ESC treated with control vehicles for 14 days (Fig. 1A, upper panel, lane 1, white arrowhead), while the ~60-kDa band disappeared in ESC treated with $E_2 + P_4$ for the same period (Fig. 1A, upper panel, lane 2).[12] In contrast, the tyrosine-phosphorylated ~56-kDa and ~64-kDa bands were detected in ESC treated for 14 days with $E_2 + P_4$, but not control vehicles (Fig. 1A, upper panel, lane 2, gray arrowheads).[12] Decidualization of the $E_2 + P_4$-treated ESC was judged by morphological changes and the production of prolactin, a typical decidualization marker (data not shown).[12]

The molecular weights of phosphotyrosinyl proteins

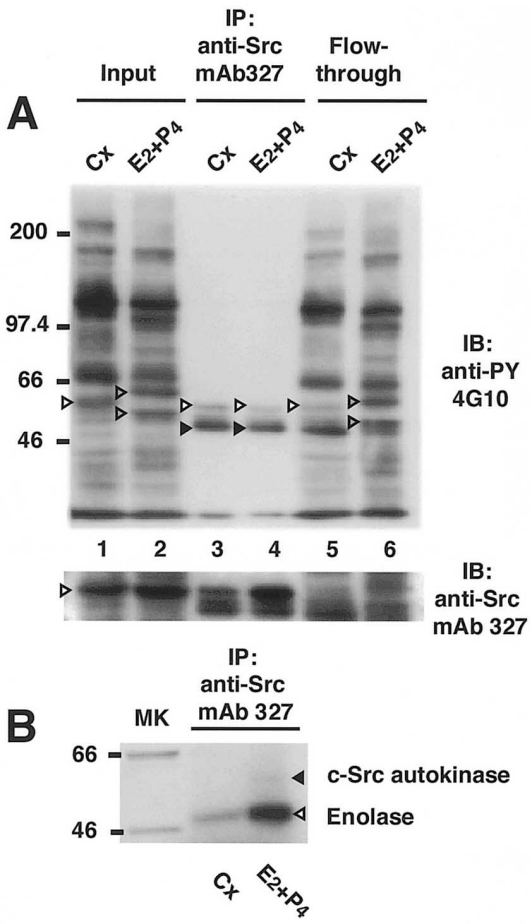

Fig. 1 Alterations in the profile of phosphotyrosinyl proteins and kinase activation of c-Src tyrosine kinase concomitant with its decreased dephosphorylation upon *in vitro* decidualization of ESC. A. Immunoblot analysis with anti-PY and anti-Src mAb 327 on the whole cell lysates, immunoprecipitates with mAb327, their flow-through derived from ESC treated with either control vehicles (Cx) or with $E_2 + P_4$ ($E_2 + P_4$) for 14 days as indicated. Closed arrowheads, IgG heavy chains. White and gray arrowheads, see the text. IP, immunoprecipitation. Lower panel, the same filter stripped and reprobed with mAb 327. B, *In vitro* kinase assay of c-Src immunoprecipitates derived from the same samples as above. MK, [^{14}C]-labeled rainbow marker. (From Maruyama T, Yoshimura Y, Yodoi J, Sabe H. Endocrinology 1999; 140: 2634. With permission, from Endocrinology, Volume 140, © 1999, by the Endocrine Society, www.endo-society.org)

affected by decidualization as shown in Figure 1A were similar to those of Src family tyrosine kinases whose activities are regulated by their own tyrosine phosphorylation[2] Src family tyrosine kinases including c-Src and Fyn are known to play a pivotal role in the differentiation of various types of cells.[2] In addition, E_2 and P_4 have been reported to activate the c-Src/p21^{ras}/Erk pathway in human breast cancer cells.[13,14] These findings collectively led us to determine whether the Src family kinases were involved in the decidualization of ESC *in vitro*.

Stripping and reprobing of the same membrane with

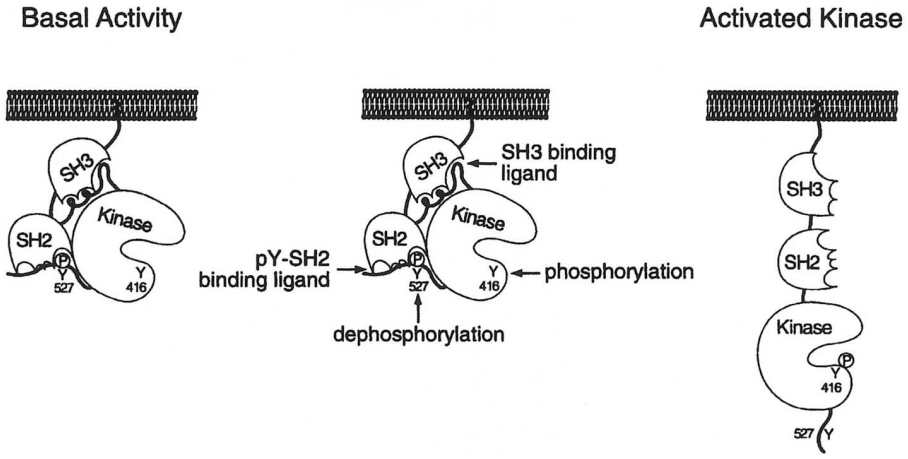

Fig. 2 Mechanisms involved in activation of Src family kinases. The left panel shows a model of the structure of inactivated Src PTKs that are phosphorylated on the C-terminal tyrosine (Y527 in this model of Src). The middle panel shows possible mechanisms involved in activation of Src PTKs. Y416 represents the autophosphorylation site in the activation loop of Src. The right panel represents a model for the activated state of Src in which the intramolecular interactions of the Src homology (SH) 3 and SH2 domains are disrupted. (From Thomas SM and Brugge JS. Annu. Rev. Cell Dev. Biol. 1997; 13: 519. With permission, from the Annual Review of Cell and Developmental Biology, Volume 13, © 1997, by Annual Reviews www.AnnualReviews.org)

anti-c-Src monoclonal antibody mAb327 revealed that c-Src was expressed at almost the same level in both untreated and treated ESC (Fig. 1A, lower panels, lanes 1 and 2).[12] We next examined employing an *in vitro* kinase assay if the kinase activity of c-Src is increased upon decidualization. As shown in Figure 1B, c-Src immunoprecipitates with mAb327 from decidualized ESC could prominently phosphorylate enolase, a substrate for c-Src, (lane 2) when compared to those from non-decidualized ESC (lane 1).[12] Intriguingly, the tyrosine phosphorylation level of the decidual c-Src was lower than that of non-decidual c-Src (Fig. 1A, upper panel, lanes 3 and 4, white arrowheads), while the same or greater amount of c-Src was immunoprecipitated from the lysates of decidualized ESC than those of non-decidualized ESC (Fig. 1A, lower panel, lanes 3 and 4).[12] Although Fyn, another Src family kinase, is also expressed in ESC, it was not activated upon decidualization (data not shown).[12] Taken together, these results suggest that c-Src became activated and dephosphorylated during *in vitro* decidualization, accompanied by alterations in tyrosine phosphorylation levels of several cellular proteins.

Possible Regulatory Mechanism(s) Underlying Decidual c-Src Activation

The kinase activity of c-Src is known to be up-regulated by dephosphorylation of its negative regulatory tyrosine residue, tyrosine 527 (Y527) (corresponding to Y530 in human), located at the C-terminus (Fig. 2).[2] To date, the plausible candidate molecules regulating the phosphorylation status of the C-terminal tail are C-terminal Src kinase (Csk) and receptor-like protein-tyrosine phosphatase-α (RPTP-α).[2] In general, Csk represses the kinase activity of c-Src through phosphorylation of Y530, while RPTP-α activates c-Src through dephosphorylation of the same residue.[2] We have here shown using immunoblot analysis with 4G10 that the kinase activation of decidual c-Src coincides with its own dephosphorylation. Osusky *et al.* have demonstrated that 4G10 can recognize a c-Src mutant whose Tyr-416 is substituted to phenylalanine (c-Src[Y416F]), but cannot fully react with either a c-Src double mutant (Y416F/Y527F) or a dephosphorylated c-Src (Y416F).[15] It has been recently reported that c-Src is dephosphorylated, as determined by immunoblotting using 4G10, with the concomitant elevation of its kinase activity in A418 cells overexpressing RPTP-α.[16] Zheng *et al.* have reported that overexpression of RPTP-α results in the activation of c-Src kinase together with its dephosphorylation of Ty-527 in rat embryo fibroblasts, as assessed by cyanogen bromide phosphopeptide mapping.[17] Taken together, our results suggest that the kinase activation of decidual c-Src may be, at least in part, due to the dephosphorylation of the negative regulatory site. This seems to be consistent with our recent data using a specific and selective monoclonal antibody against the active form of human c-Src whose Y530 is dephosphorylated (unpublished data).

Alternatively, c-Src is known to be able to couple with a number of receptor protein tyrosine kinases such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), colony-stimulating factor 1 (CSF-

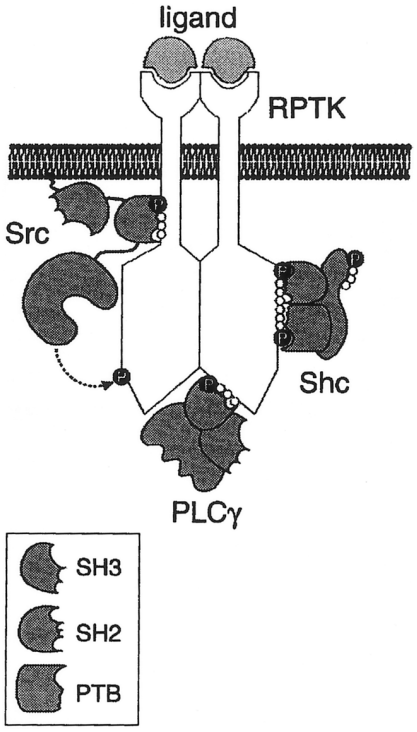

Fig. 3 Interaction of RPTKs with Src and several representative SH2-containing signaling proteins. Src interacts with tyrosine phosphorylated motifs in RPTKs through its SH2 domain. Other proteins, such as Shc and PLC shown here, also bind to RPTKs through related SH2 domains. Src can also phosphorylate tyrosine residues on the receptor. The position of the Src SH2 binding motif at the juxtamembrane location represents the location of the Src binding sites on the PDGF receptor. (From Thomas SM and Brugge JS. Annu. Rev. Cell Dev. Biol. 1997; 13: 542. With permission, from the Annual Review of Cell and Developmental Biology, Volume 13, © 1997, by Annual Reviews www.AnnualReviews.org)

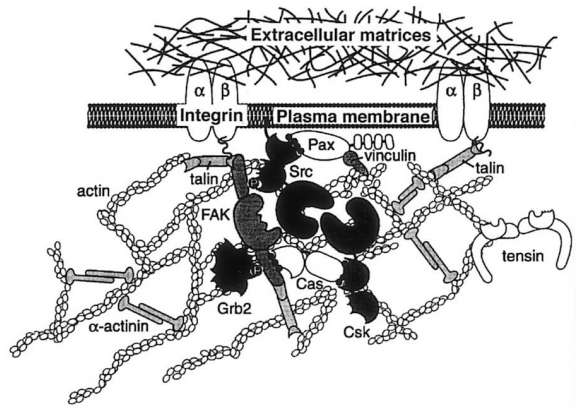

Fig. 4 Src interaction with components of focal adhesion complexes. This figure was designed to indicate that Src associates with focal adhesions following engagement of integrins by the extracellular matrix. The intermolecular interactions displayed are hypothetical, based on known interactions *in vitro* or co-immunoprecipitation from cell lysates and represent only a small number of proteins within these complexes. (Adapted from Thomas SM and Brugge JS. Annu. Rev. Cell Dev. Biol. 1997; 13: 531. With permission, from the Annual Review of Cell and Developmental Biology, Volume 13, © 1997, by Annual Reviews www.AnnualReviews.org)

1), and insulin-like growth factor (IGF) receptors upon their binding to the relevant ligands (Fig. 3).[2] In addition, many G-protein coupled receptors and cytokine receptors including angiotensin II, bombesin, bradykinin, vasopressin, platelet activating factor (PAF), interleukin-11 (IL-11), prolactin, and oncostatin M can also associate with c-Src.[2] These transmembrane receptors recruit and activate c-Src as well as the other Src family kinases upon ligand binding, thereby transmitting the extracellular stimuli to the intracellular signal.[2] Importantly, those c-Src activating factors are also locally produced by the endometrium and decidua.[4–6] Thus, this knowledge collectively substantiates an idea that several decidual paracrine/autocrine factors might activate signaling pathways involving c-Src.

Reactive oxygen species (ROS), generated by reduction-oxidation (redox) reactions, are also known to activate c-Src.[18] Stimulation by some growth factors such as EGF and PDGF generates intracellular H_2O_2-derived ROS that in turn mediate the signal transduction downstream of the corresponding receptors.[19] Given that the expression of several redox-regulatory proteins including superoxide dismutase and thioredoxin is induced during decidualization,[20–22] it is conceivable that those redox-active proteins together with growth factors and cytokines might coordinately regulate the kinase activity of decidual c-Src.

Downstream Target Proteins of Decidual c-Src

To gain a clue for the c-Src-mediated signaling pathway in decidualization, we then examined the tyrosine phosphorylation of paxillin and focal adhesion kinase (FAK). They are deeply involved in the integrin-mediated signal transduction through focal adhesion assembly, serving as substrates for the Src family tyrosine kinases (Fig. 4).[2,23] As shown in Fig. 5, the whole pattern of tyrosine phosphorylation of cellular proteins was changed during E2 + P4-induced decidualization (lanes 1 and 2) in a similar way as demonstrated in Fig. 1A (upper panel, lanes 1 and 2).[24] Despite the alterations in the entire pattern of tyrosine phosphorylation, there was little difference in the tyrosine phosphorylation levels of both the paxillin (Fig. 5, lanes 3 and 4, asterisks) and 125-kDa FAK (lanes 5 and 6, sharps) immunoprecipitates between non-decidualized and decidualized ESC.[24]

The formation of focal adhesions are prerequisite for the integrin-mediated signaling pathways respon-

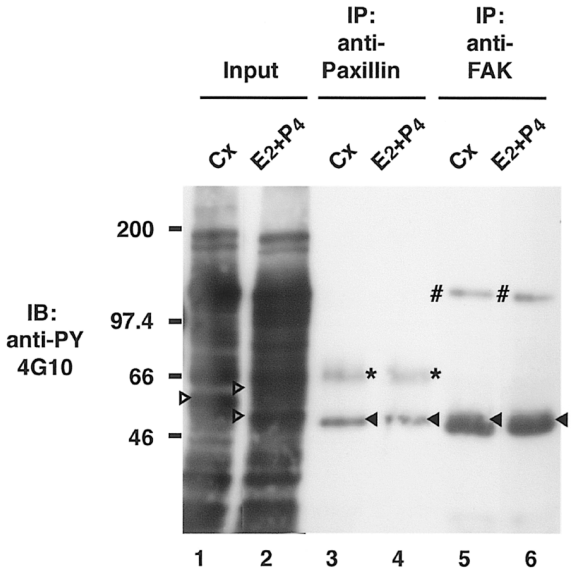

Fig. 5 No marked changes in tyrosine phosphorylation of FAK and paxillin upon decidualization. Immunoblot data using anti-PY antibody on whole cell lysates derived from ESC treated with either control vehicles (Cx) or with $E_2 + P_4$ ($E_2 + P_4$) for 14 days and immunoprecipitates with anti-FAK or anti-paxillin antibody as indicated. Closed arrowheads, IgG heavy chains. *, tyrosine-phosphorylated paxillin. ♯, tyrosine-phosphorylated FAK. (Adapted from Maruyama T, Yoshimura Y, Sabe H. Endocrinology 1999; 140: 5984. With permission, from Endocrinology, Volume 140, © 1999, by the Endocrine Society, www.endo-society.org)

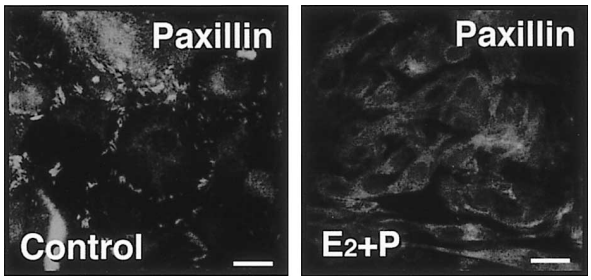

Fig. 6 Disappearance of the focal contacts in the decidualization-induced multicellular nodules Immunofluorescence micrographs using anti-paxillin antibody of ESC treated with either control vehicles (Control) or with $E_2 + P_4$ ($E_2 + P$) for seven days. Bars, 25 μm. (Adapted from Maruyama T, Yoshimura Y, Sabe H. Endocrinology 1999; 140: 5984. With permission, from Endocrinology, Volume 140, © 1999, by the Endocrine Society, www.endo-society.org)

sible for cell growth, differentiation, migration, transformation, and contractility.[23] To address the question why tyrosine phosphorylation of FAK and paxillin was not markedly changed despite decidual c-Src activation, we next analyzed the subcellular localization of the focal adhesion proteins focusing on the characteristic decidualization-induced multicellular nodules. ESC in the control cultures clearly possessed focal contacts, as assessed by the immunostaining of paxillin (Fig. 6, left panel).[24] In contrast, in the decidualization-induced multicellular nodules, no discrete accumulations of paxillin staining could be detected at borders of adjoining cells or at distinct focal adhesion plaques; but rather paxillin was diffusely localized in the cytosol (Fig. 6, right panel).[24]

The orderly recruitment and complex formation of focal adhesion proteins are crucial for the actin-based cytoskeletal organization.[23] We further investigated the subcellular distribution of actin stress fibers in the multicellular nodules where the formation of the focal adhesion assembly was impaired. Double-labeled immunostaining of ESC using FITC-conjugated phalloidin and anti-vinculin antibody, visualized by rhodamine-conjugated secondary antibody, showed that the undifferentiated and flattened ESC in the control cultures clearly possessed focal adhesion contacts containing vinculin, one of the focal adhesion proteins (Fig. 7, left upper panel).[24] Moreover, there were well-stretched actin stress fibers (Fig. 7, left middle panel), linking paired focal adhesion contacts (left lower panel).[24] However, well-organized F-actin could not be detected but was rather located at the cell periphery within decidualization-induced multicellular nodules (Fig. 7, right panel).[24] We also obtained similar findings with paxillin (data not shown).[24] Together with the elevation of the c-Src kinase activity during *in vitro* decidualization, our present data suggest that c-Src may activate a different signaling pathway(s), which does not involve FAK and paxillin, in decidualized ESC. Furthermore, dissociation of the focal adhesion complex together with the breakdown of the actin-based cytoskeleton might contribute to both functional and morphological decidualization.

Role of c-Src in Decidual Transformation

c-Src primarily co-localizes with markers of endosomal membranes and associates with specialized secretory vesicles in some cell types,[25,26] implicating c-Src as a possible regulator of exocytosis.[27,28] Considering the high secretory potential of decidual cells,[4] it is possible that c-Src may regulate secretion of many decidualization-associated bioactive substances. Alternatively, v-Src, a constitutively kinase-active variant of c-Src, possesses an oncogenic transformation activity including cell rounding and detachment.[2,29] Also, the kinase activation of c-Src has been implicated in the regulation of the actin-based cytoskeletal organization and focal adhesion assembly.[2,29] Therefore, it is conceivable that c-Src might play a role in a unique decidual transformation from fibroblastic stromal cells into metabolically active, round, and enlarged decidual cells. Elucidation of the relevance of c-Src activation to the decidual function awaits further studies.

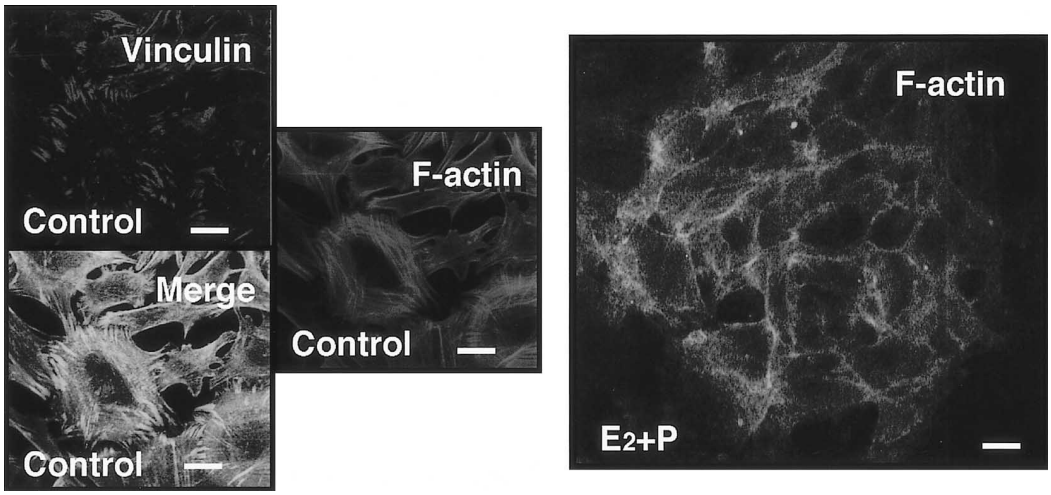

Fig. 7 Disorganization of the actin-based cytoskeleton in decidualization-induced multicellular nodules. ESC treated with either control vehicles (Control) or with $E_2 + P_4$ ($E_2 + P$) were double-stained for F-actin using FITC-conjugated-phalloidin and for vinculin using anti-vinculin antibody that was subsequently visualized by rhodamine-conjugated secondary antibody. Bars, 25 μm. (Adapted from Maruyama T, Yoshimura Y, Sabe H. Endocrinology 1999; 140: 5984. With permission, from Endocrinology, Volume 140, © 1999, by the Endocrine Society, www.endo-society.org)

Analogy to the Differentiation of Other Cells Types

Lastly, since c-Src is a proto-oncogene product, its dysregulated overexpression and activation are thought to be closely associated with cell growth and tumorigenesis rather than differentiation.[2,29] However, quite a few studies have implicated c-Src and its activation in the differentiation of several types of cells such as osteoclasts,[30] keratinocytes,[31] and neuronal cells.[32,33] Intriguingly, 1,25-dihydroxyvitamin D3, which is required for commitment of macrophage progenitors to osteoclast differentiation, has been shown to induce the kinase activation of c-Src, but not the expression, together with dephosphorylation of c-Src.[29] Thus, it is likely that there may be a common mechanism involving c-Src activation for some types of differentiation including decidualization.

Concluding Remarks

As mentioned previously, many decidualization-induced bioactive substances such as PDGF, EGF, CSF-1, IGF, IL-11, angiotensin II, bradykinin, PAF, prolactin, and oncostatin M behave as ligands for the transmembrane receptors that can couple with and activate c-Src, raising the possibility that decidual c-Src might be a point of convergence in the action of those factors. In this regard, it is tempting to speculate that c-Src may act as the master regulator of decidual function through selectively and timely transducing signals from the extracellular stimuli. Our current data will not only broaden our understanding of endometrial physiology but also contribute to the development of new drugs and treatments for female infertility, recurrent pregnancy loss, and contraception to target the decidua-specific tyrosine phosphorylation signaling cascade(s).

Acknowledgments: We thank Drs. H. Sabe, J. Yodoi, T. Takamatsu, and H. Kanzaki for their valuable comments and technical advice. We acknowledge the technical and secretarial assistance of Ms. R. Sakurai and S. Kuwabara. This work has been supported, in part, by grants from the Ministry of Education, Science, and Culture of Japan (B:12470348), Keio Gijuku Academic Development Funds, and grants from the Keio Health Counseling Center.

References

1. Hunter T: The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease. Philos Trans R Soc Lond B Biol Sci 1998; 353: 583–605
2. Thomas SM, Brugge JS: Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 1997; 13: 513–609
3. Noyes RW, Hertig AT, Rock J: Dating the endometrial biopsy. Fertil Steril 1950; 1: 3–25
4. Gurpide E, Tabanelli S, Tang B: Human endometrial stromal cells. In: Genazzani AR, Petraglia F, eds, Hormones in Gynecological Endocrinology, Plenary sessions, 3rd World Congress of Gynecological Endocrinology, Papers, Parthenon, 1992; 717–724
5. Tabibzadeh S: Human endometrium: an active site of cytokine production and action. Endocr Rev 1991; 12: 272–290
6. Giudice LC: Growth factors and growth modulators in human uterine endometrium: their potential relevance to reproductive medicine. Fertil Steril 1994; 61: 1–17
7. Huang JR, Tseng L, Bischof P, Janne OA: Regulation of prolactin production by progestin, estrogen, and relaxin in human endometrial stromal cells. Endocrinology 1987; 121: 2011–2017

8. Irwin JC, Kirk D, King RJ, Quigley MM, Gwatkin RB: Hormonal regulation of human endometrial stromal cells in culture: an *in vitro* model for decidualization. Fertil Steril 1989; 52: 761–768
9. Tang B, Guller S, Gurpide E: Cyclic adenosine 3′,5′-monophosphate induces prolactin expression in stromal cells isolated from human proliferative endometrium. Endocrinology 1993; 133: 2197–2203
10. Telgmann R, Maronde E, Tasken K, Gellersen B: Activated protein kinase A is required for differentiation-dependent transcription of the decidual prolactin gene in human endometrial stromal cells. Endocrinology 1997; 138: 929–937
11. Brar AK, Frank GR, Kessler CA, Cedars MI, Handwerger S: Progesterone-dependent decidualization of the human endometrium is mediated by cAMP. Endocrine 1997; 6: 301–307
12. Maruyama T, Yoshimura Y, Yodoi J, Sabe H: Activation of c-Src kinase is associated with *in vitro* decidualization of human endometrial stromal cells. Endocrinology 1999; 140: 2632–2636
13. Migliaccio A, Di Domenico M, Castoria G, de Falco A, Bontempo P, Nola E, Auricchio F: Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. EMBO J 1996; 15: 1292–1300
14. Migliaccio A, Piccolo D, Castoria G, Di Domenico M, Bilancio A, Lombardi M, Gong W, Beato M, Auricchio F: Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J 1998; 17: 2008–2018
15. Osusky M, Taylor SJ, Shalloway D: Autophosphorylation of purified c-Src at its primary negative regulation site. J Biol Chem 1995; 270: 25729–25732
16. Harder KW, Moller NP, Peacock JW, Jirik FR: Protein-tyrosine phosphatase alpha regulates Src family kinases and alters cell-substratum adhesion. J Biol Chem 1998; 273: 31890–31900
17. Zheng XM, Wang Y, Pallen CJ: Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 1992; 359: 336–339
18. Droge W: Free radicals in the physiological control of cell function. Physiol Rev 2002; 82: 47–95
19. Finkel T: Redox-dependent signal transduction. FEBS Lett 2000; 476: 52–54
20. Maruyama T, Kitaoka Y, Sachi Y, Nakanoin K, Hirota K, Shiozawa T, Yoshimura Y, Fujii S, Yodoi J: Thioredoxin expression in the human endometrium during the menstrual cycle. Mol Hum Reprod 1997; 3: 989–993
21. Maruyama T, Sachi Y, Furuke K, Kitaoka Y, Kanzaki H, Yoshimura Y, Yodoi J: Induction of thioredoxin, a redox-active protein, by ovarian steroid hormones during growth and differentiation of endometrial stromal cells *in vitro*. Endocrinology 1999; 140: 365–372
22. Sugino N, Kashida S, Takiguchi S, Nakamura Y, Kato H: Induction of superoxide dismutase by decidualization in human endometrial stromal cells. Mol Hum Reprod 2000; 6: 178–184
23. Burridge K, Chrzanowska-Wodnicka M: Focal adhesions, contractility, and signaling. Annu Rev Cell Dev Biol 1996; 12: 463–518
24. Maruyama T, Yoshimura Y, Sabe H: Tyrosine phosphorylation and subcellular localization of focal adhesion proteins during *in vitro* decidualization of human endometrial stromal cells. Endocrinology 1999; 140: 5982–5990
25. Grandori C, Hanafusa H: p60c-src is complexed with a cellular protein in subcellular compartments involved in exocytosis. J Cell Biol 1988; 107: 2125–2135
26. Linstedt AD, Vetter ML, Bishop JM, Kelly RB: Specific association of the proto-oncogene product pp60c-src with an intracellular organelle, the PC12 synaptic vesicle. J Cell Biol 1992; 117: 1077–1084
27. Barnekow A, Jahn R, Schartl M: Synaptophysin: a substrate for the protein tyrosine kinase pp60c-src in intact synaptic vesicles. Oncogene 1990; 5: 1019–1024
28. Oddie KM, Litz JS, Balserak JC, Payne DM, Creutz CE, Parsons SJ: Modulation of pp60c-src tyrosine kinase activity during secretion in stimulated bovine adrenal chromaffin cells. J Neurosci Res 1989; 24: 38–48
29. Abram CL, Courtneidge SA: Src family tyrosine kinases and growth factor signaling. Exp Cell Res 2000; 254: 1–13
30. Chappel J, Ross FP, Abu-Amer Y, Shaw A, Teitelbaum SL: 1,25-dihydroxyvitamin D3 regulates pp60c-src activity and expression of a pp60c-src activating phosphatase. J Cell Biochem 1997; 67: 432–438
31. Zhao Y, Sudol M, Hanafusa H, Krueger J: Increased tyrosine kinase activity of c-Src during calcium-induced keratinocyte differentiation. Proc Natl Acad Sci USA 1992; 89: 8298–8302
32. Cartwright CA, Simantov R, Kaplan PL, Hunter T, Eckhart W: Alterations in pp60c-src accompany differentiation of neurons from rat embryo striatum. Mol Cell Biol 1987; 7: 1830–1840
33. Bjelfman C, Meyerson G, Cartwright CA, Mellstrom K, Hammerling U, Pahlman S: Early activation of endogenous pp60src kinase activity during neuronal differentiation of cultured human neuroblastoma cells. Mol Cell Biol 1990; 10: 361–370