The Molecular Pharmacology of Estrogen Receptor Modulators: Implications for the Treatment of Breast Cancer

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ABSTRACT

In addition to physiologic activities in the reproductive, skeletal, and central nervous systems, estrogens have been shown to play important roles in the aberrant cell proliferation observed in breast and reproductive tract cancers. Not surprisingly, pharmaceuticals that target different steps in the estrogen signal transduction pathway have found widespread use in the treatment of a wide variety of estrogen-linked disorders. The goal of this review is to outline what is known about the molecular pharmacology of the estrogen receptor and discuss how this information can be used to guide selection of drugs for a particular therapeutic application, and identify new targets where pharmaceutical exploitation could yield novel therapeutics.

THE MOLECULAR MECHANISM OF ACTION OF ESTROGEN

In breast cancer, agents that interfere with the activation of the estrogen receptor (ER) by blocking estradiol synthesis or inhibiting the interaction of estrogen with its receptor have become primary interventions in both the adjuvant and metastatic breast cancer settings and have a developing role as chemopreventives (1–3). However, although the current classes of drugs have had a very positive impact on the morbidity and mortality associated with breast cancer, it has become clear from studies of the molecular pharmacology of ER that additional exploitation of this target will yield drugs with improved therapeutic utility (1, 2). A brief overview of the mechanism of action of estrogen will provide the reader with an appreciation of the complexities of this signal transduction pathway and will highlight the emerging opportunities for drug discovery in this field.

The biological actions of estrogens are mediated by two genetically and functionally distinct receptors, ERα and ERβ, which share similar, though distinct, cellular expression profiles in target cells (4). Upon binding an agonist, these receptors spontaneously form ERα/ERβ heterodimers in cells expressing both receptor subtypes or homodimeric complexes in cells expressing a single subtype (5). The resulting ER-dimer is then capable of interacting with specific regulatory sequences within target gene promoters exerting either a positive or negative effect on gene transcription. Whereas both receptor subtypes are capable of activating transcription, ERα seems to be a more robust activator than ERβ (5). Not surprisingly, therefore, in cells where both receptors are expressed, it has been shown that ERβ moderates the agonist activity of estradiol (6). Finally, although ERα and ERβ share mechanistic similarities, they play different roles in estrogen action, a conclusion that is supported by the distinct phenotypes observed in mice in which either or both subtypes have been genetically disrupted, and the observation that ERβ-specific agonists function as therapeutically effective treatments in animal models of inflammatory bowel disease and rheumatoid arthritis but unlike estradiol (not selective), they do not manifest agonist activity in the reproductive tract, bone, or mammary glands (7).

Agonist-activated ER can also function as a transcriptional repressor by inhibiting the activity of transcription factors such as nuclear factor κB (8). This inhibition results from the physical interaction of the agonist-bound ER with the p65 subunit of activated nuclear factor κB. This inhibitory activity of ER explains, in part, the antiinflammatory actions of estrogens in brain and the cardiovascular system and contributes to the antiresorptive actions of estrogens in bone (9, 10). In addition to direct effects on transcription, it has been shown *in vitro* that estrogens, acting through either ERα or ERβ, can also participate in nongenomic, extranuclear signaling events (11). Although consensus has not been reached as to the mechanism or physiologic relevance of these latter activities, it is clear that there are responses to estrogens that occur in the cytoplasm, or at the cell membrane of target cells, that do not impact gene expression directly. Among these observed responses, the activation of mitogen-activated protein kinase, regulation of calcium transients, and activation of epithelial nitric oxide synthase in membrane caveoli are the most intriguing (12, 13).

THE MOLECULAR DETERMINANTS OF ER PHARMACOLOGY

Until recently, it was considered that the pharmacology of ER was relatively uncomplicated. It was proposed that agonists functioned as molecular switches that bound and converted ER from an inactive to an active form. By inference, therefore, all agonists were considered to be qualitatively the same. Conversely, antagonists were believed to function by competitively inhibiting agonist binding, thereby freezing the receptor in an apo state (14). Thus, within the confines of this model, compounds like tamoxifen were classified as ''antiestrogens'', as they competitively inhibited agonist binding to ER and opposed the actions of estrogens in most target tissues. Of significance, they were shown to oppose the mitogenic actions of estrogen in the breast and to inhibit significantly the growth of ER-positive tumors (15). However, it is interesting that as early as 1967, there was evidence to suggest that tamoxifen could

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function as an agonist in some tissues at the same time functioning as an antagonist in others within the same animal (16). The significance of this mixed agonist activity was not realized until 1992, when a key study showed, in a placebo-controlled trial in postmenopausal breast cancer patients undergoing adjuvant chemotherapy, that tamoxifen was an effective ER agonist in bone (17). This seminal finding led to the birth of a new class of ER ligands, selective estrogen receptor modulators (SERM), compounds whose relative agonist and antagonist activities varied between target tissues. These findings begged a reevaluation of the classical models of ER action and its inferred pharmacology.

An understanding of the molecular basis of SERM activity has emerged with the discovery of receptor interacting proteins and the subsequent demonstration that their expression levels and biological activities could differ between cells (Fig. 1). Functionally, there are two main classes of receptor interacting proteins: coactivators, which enhance, and corepressors, which repress, transcriptional activity. Coactivators function by nucleating multiprotein complexes at target gene promoters, which facilitate chromatin decondensation and enhanced transcription by catalyzing histone acetylation. Corepressors function in reverse by recruiting histone deacetylases, which results in a local condensation of chromatin and dampening of transcriptional activity. Not surprisingly, it has been shown that the transcriptional activity of different ER-ligand complexes depends on which coactivators and corepressors are available in target cells. This work has led to the development of the "coactivator hypothesis" and a plausible explanation as to how the same ligand can manifest different biological activities in different cells (18). However, in and of itself, differential cofactor expression cannot explain why chemically similar ligands, like tamoxifen and raloxifene, for example, can have different activities in the same cell. This would not be expected if the primary determinant of ER pharmacology were merely coactivator availability. Resolution of this issue came from the observation that ER does not exist merely in an "on" or "off" conformation within cells but rather the overall topography of the receptor surface is influenced by the nature of the bound ligand (19–21). It was further shown that receptor conformation influences the cofactor preferences for the receptor. Thus, it is likely that tamoxifen functions as an agonist in bone because the conformation that the ER adopts in the presence of this ligand allows it to hook up with factors that enable it to activate transcription. It is inferred that in breast, a similarly conformed receptor functions as an antagonist as it is unable to find a coactivator partner (19, 21–25). Final proof of the coactivator/conformation hypothesis awaits the identification of the specific factors that ER engages in different cells and a subsequent demonstration of the functional consequences of each of these interactions.

In addition to absolute coactivator expression levels, it has also been shown that ER pharmacology can also be influenced by cell signaling pathways that impinge upon and modulate the activity of different receptor/cofactor complexes (26). Under normal physiologic conditions, these alternate pathways of activation are probably involved in the fine-tuning of ER action (27). However, it is clear that in certain pathologic conditions, they could dominate and could even obviate the need for a ligand (28). Importantly, manipulation of the activity of several different signaling pathways has been shown to impact the relative agonist/antagonist activity of SERMs like tamoxifen (29).

In summary, it is clear that the molecular pharmacology of ER is extremely complex, being influenced by (*a*) relative expression of ERα and ERβ, (*b*) ligand effects on receptor structure, (*c*) availability of cofactors, and (*d*) the activity of signaling pathways that impinge upon the activated receptor.

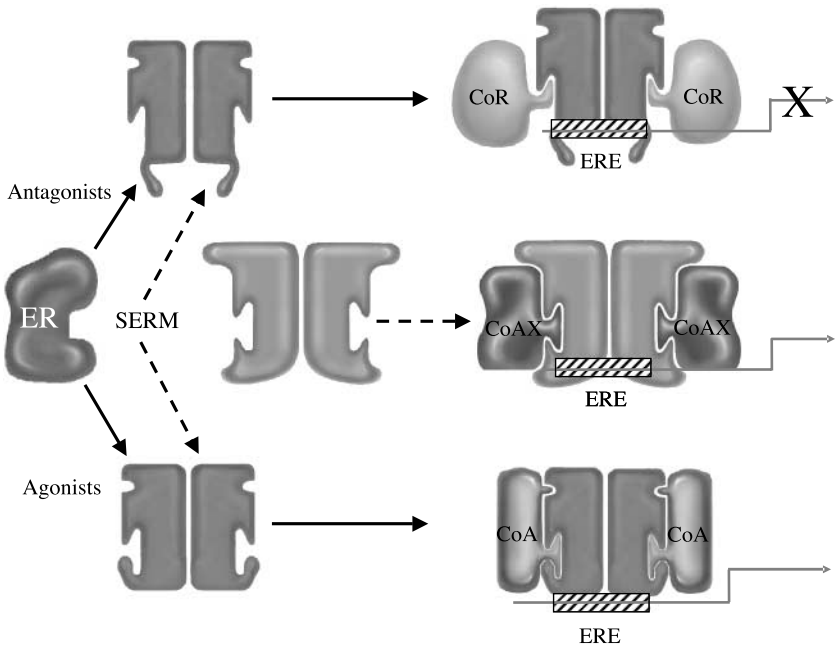

Fig. 1 An updated model of ER action may help to explain the activity of ER modulators. Upon binding an agonist or an antagonist, ER undergoes a conformational change that permits its spontaneous dimerization and facilitates the subsequent interaction of the dimer with estrogen response elements (ERE) located within target genes. Two genetically distinct ERs have been identified, ERα and ERβ, which have the potential to form homodimers or heterodimers in cells where both subtypes are expressed. It has recently been determined that different ligands can have different effects on ER structure. The functional consequences of different ligand-induced conformational changes were revealed with the discovery of receptor coactivators (CoA) and corepressors (CoR). Coactivators (CoAX = coactivator "X") interact with agonist-activated ER and facilitate transcriptional activation, whereas corepressors interact with antagonist-activated receptor and help to maintain it in a quiescent state. SERMs permit the receptor to adopt a structure that is intermediate between that observed following the binding of agonists or antagonists.

Within this context, it can be appreciated why structurally similar ER ligands can have dramatically different effects in target cells.

THE MECHANISTIC DIFFERENCES BETWEEN SERMs AND ANTIESTROGENS HAVE PRACTICAL IMPLICATIONS

The reclassification of tamoxifen as a SERM as opposed to an antiestrogen has led to a discussion as to what constitutes an ER antagonist. The term antagonist is usually used to describe a compound that impedes the activity of an activating ligand while having a neutral effect on the physical state of the receptor. However, because all known ER ligands facilitate the displacement of the receptor from heat shock proteins, they actually "activate" the receptor (30). Thus, the term antagonist must be reserved for compounds that displace ER from inhibitory heat shock protein complexes but which do not facilitate coactivator recruitment. One approach to identify pure antagonists is to screen for compounds that induce a receptor conformation that is unable to recruit any coactivators. However, given that ER has been shown to interact with over 50 different proteins using several different surfaces, it is clear why compounds of this type have not yet been described (31). A second mechanism to achieve pure antagonism is to identify compounds that destabilize the receptor and facilitate its degradation. The latter mechanism seems to explain how compounds like fulvestrant function as "pure antagonists" (32). We and others have determined that upon binding fulvestrant, ER adopts a conformation that is distinct from that observed when an agonist or a SERM is bound (19, 21, 25, 33). Each uniquely conformed receptor undergoes a distinct pattern of ubiquitination, the consequence of which is an alteration in receptor stability; tamoxifen stabilizes whereas fulvestrant destabilizes the receptor (32). Because of this unique mode of action, fulvestrant and related compounds have been described as selective estrogen receptor down-regulators (SERD), a subclass of antagonists. Fulvestrant has been approved for the treatment of metastatic breast cancer. SERDs seem to have the most profound effect in the treatment of tamoxifen-refractory tumors where some trials have reported a > 60% response rate (34). This might make sense when the mechanism proposed to explain *de novo* and acquired tamoxifen resistance in ER-positive tumors is considered.

Several distinct mechanisms have been described to explain resistance to first-line tamoxifen therapy, most of which involve a switch in tamoxifen's role from antagonist to agonist. As mentioned, tamoxifen functions as an antagonist because it allows the receptor to adopt a conformation that significantly reduces its affinity for the coactivators that are normally recruited to agonist-activated ER (21). However, it has been shown *in vitro* and in animal models that this lowered affinity can be offset by elevated expression of specific coactivators (31, 35). The importance of these data was underscored by the observations that overexpression of ER coactivators, especially SRC-3 (AIB1), have been observed in a large number of breast cancers and that AIB1's overexpression, together with overexpression of HER2/neu, correlate clinically with an increased incidence of tamoxifen resistance (28, 36). Furthermore, although the primary coactivator binding pockets are disrupted or distorted by the tamoxifen-induced alterations in ER structure, it has been shown that secondary sites are presented that could facilitate ectopic interactions of the receptor with cofactors with which it may not normally couple. Thus, resistance could arise either as a consequence of the outgrowth of cells which *de novo* express a cofactor that can support tamoxifen agonist activity, and/or an epigenetic change which leads to the enhanced expression of an appropriate cofactor. A third, nonexclusive possibility is that activation of mitogen-activated protein kinase by a variety of growth factors and cytokines can potentiate tamoxifen agonist activity through direct actions on either the coactivators or the receptors themselves. Regardless of which mechanism, or combination of mechanisms, of resistance is operative in a given tumor, it is clear that all are ER-dependent activities and that removal of the receptor is the best way of achieving total blockade of signaling. It should be appreciated from this discussion why SERDs are very effective in treating ER-positive tamoxifen-resistant tumors but manifest similar activities to tamoxifen as primary therapies.

THE MECHANISTIC DIFFERENCES BETWEEN SERMs AND SERDs HAVE IMPORTANT CLINICAL IMPLICATIONS

Whereas SERDs have been shown to be as effective as aromatase inhibitors in the treatment of advanced breast cancer, they are no more and possibly even less effective than tamoxifen as a first-line therapy (37). Intuitively, this does not seem to make sense because eliminating the receptor would presumably be the most effective way of suppressing estrogen action in target cells. However, we are now beginning to understand at a molecular level why SERMs and SERDs are not functionally equivalent. To understand these important differences, we must return to a discussion of the complexities of tamoxifen action. The interaction of tamoxifen with ER induces a conformational change in the receptor that facilitates its interaction, directly or indirectly, with target gene promoters. However, in most cells the tamoxifen/ER complex is unable to recruit coactivators and is thus transcriptionally inactive. Moreover, there is considerable evidence to suggest that in the tamoxifen-bound state ER can recruit corepressors, factors that help to maintain target genes in a suppressed state (38). When associated with target gene promoters, the tamoxifen-bound ER also inhibits gene transcription by competitively inhibiting the interaction of other nuclear receptors, like estrogen-related receptor and LRH-1, and the peroxisome proliferator-activated receptors (PPAR) with estrogen response elements within target gene promoters. Of note, it has been observed that most breast tumors express estrogen-related receptor α and PPARγ, and the former is an important prognostic factor in ER-positive breast tumors (39–41). Thus, by having the inactive tamoxifen-activated ER on promoters, they are insulated from the positive transcriptional activities of other estrogen response element binders. Obviously, these latter inhibitory activities are not manifest in SERD-treated cells or tumors where the receptor is degraded. Considering these mechanistic differences, it becomes clear why there may be no advantage to using a SERD over a SERM as a first-line breast cancer chemotherapeutic or chemopreventive.

THE NEXT GENERATION OF ER MODULATORS

The terms SERMs and SERDs describe families of chemicals with similar pharmacologic properties or activities (Fig. 2). However, given the complexity of the ER signal transduction pathway, it should be apparent that some compounds might exhibit the characteristics of both SERMs and SERDs. A case in point is the compound GW5638/DPC 974, a novel antiestrogen that is currently being evaluated in patients with tamoxifen-resistant metastatic breast cancer (42). This mixed function SERM/SERD has been shown to function as an estrogen (SERM-like) in the skeletal and cardiovascular systems whereas it opposes the actions of estrogen in the uterus (42–44). More importantly, however, in animal models, this agent inhibits the growth of tumors that are resistant to (stimulated by) tamoxifen (42). Exploration of the molecular mechanism of action of GW5638 revealed that it decreases ER half-life, although not as dramatically as fulvestrant, and that it allows ER to adopt a unique conformation which blocks the presentation of surfaces on ER required for tamoxifen agonist activity (21). This explains why tumors that are resistant to tamoxifen are not cross-resistant to GW5638, whereas cross-resistance is noted between SERMs that allow ER to adopt a "tamoxifen-like structure." Although interesting from a mechanistic point of view, the therapeutic benefit of mixed function SERMs/SERDs remains to be determined.

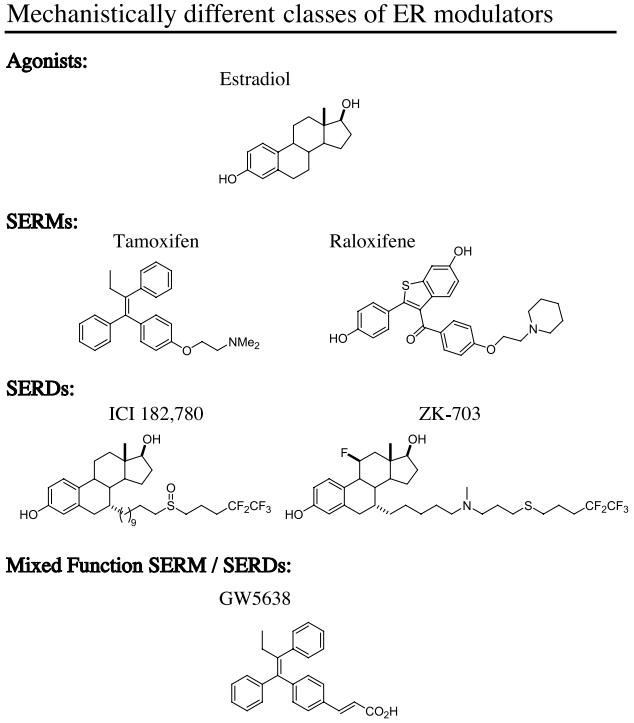

Fig. 2 Mechanistically different classes of ER modulators. Until recently, it was considered that the ligands for ER could be described as either agonists or antagonists. However, as our understanding of ER pharmacology has advanced, it is clear that there are no compounds which fit the pharmacologic definition of "antagonists." SERMs can function as either agonists or antagonists depending on the coactivator balance in a given target cell. SERDs, on the other hand, manifest antagonist activities by facilitating receptor turnover.

The observation that the overall conformation of ER is influenced by the nature of the bound ligand and that the primary regulator of cofactor recruitment is receptor conformation indicates that it will be possible to develop new classes of modulators with very different pharmacologic activities. Tamoxifen was initially developed as an antiestrogen in an early contraception program long before the concept of tissue specificity was conceived. Fortuitously, further exploration of tamoxifen's properties has helped us to understand the complexities of ER action. Likewise, fulvestrant and its early incarnation ICI164,384 were developed at a time when it was not appreciated that antiestrogens could be mechanistically distinct and when resistance was believed to occur in a mechanism-unrelated manner (45). It is likely, guided by our current understanding of the molecular determinants of ER pharmacology, that a new wave of ER modulators with improved specificity will emerge which will have attributes making them more suitable for use in the treatment and prevention of breast cancer. An interesting approach that is likely to bear fruit will come from the identification of specific coactivators associated with tamoxifen resistance and the subsequent development of screens for modulators that regulate the interaction of ER with these factors. Similarly, compounds that favor specific ER/cofactor interactions that have beneficial effects in bone and the cardiovascular systems but have a neutral or antagonist activity in the breast are highly sought after for use in the adjuvant and prevention settings. In short, we are moving very close to the day where a proteomic profile of a tumor will determine the best SERM, SERD or other endocrine therapy that will yield maximal benefit in the clinic.

FINAL COMMENTS

Until recently, there was a notion among many that the advent of aromatase inhibitors was going to obviate the need for ER ligands in the management of breast cancer. Predictably, whereas aromatase inhibitors are a significant advance, they also have problems that limit their use. Among the most significant is the long-term impact of estrogen deprivation (osteoporosis, dyspareunia, etc.). In addition, aromatase-treated cells have been shown to develop hypersensitivity to low levels of estrogens and xenobiotic estrogenic substances, the latter which, under normal circumstances, have a marginal biological impact (46, 47). There is no doubt that targeting the ER signaling pathway at different points has provided many useful breast cancer therapeutics. However, out of our developing understanding of the complexities of ER signaling, opportunities will arise for the development of new and improved modulators that may replace current treatments or operate alongside them in the treatment of this chronic disease.

OPEN DISCUSSION

Dr. Douglas Yee: Your comments would suggest a CXCR4 antagonist would be a good way to proceed. Have you looked at that in your models?

Dr. Donald McDonnell: We are doing those studies now. There are several such antagonists that are orally available right now, but they are difficult to obtain because the pharmaceutical companies are not really interested in getting them tested for breast cancer as these are being tested for AIDS.

Dr. Richard Santen: In terms of the RXR blockade of LRH-1, one probably needs to distinguish between prevention and treatment of breast cancer. For those of you who aren't aficionados of aromatase, there are nine different upstream exons that stimulate production of aromatase; each one of these has different promoters, and in breast cancer there is a shift from I.4 to I.2. Using this approach for treatment of breast cancer would probably get the tumor to select the promoter that was not responsive to the inhibitor. However, in the prevention setting, where the preadipocyte aromatase expression is blocked by the RXR, probably the strategy would be very successful.

Dr. McDonnell: I am not taking a position either way because we don't actually have data. The only study that has been done looking at RXR agonists was in the prevention setting or early treatment stage, which is basically Rich Heyman's work [Cancer Res 1996;56:5566–70]. When we treat these adipocytes with RXR antagonists, we don't see any aromatase mRNA produced, but we are only doing this in the short term, so you might be right, there may be a switch later on.

Dr. Santen: I think the switch is probably part of the neoplastic process itself.

Dr. McDonnell: That is Evan Simpson's hypothesis: that as cells become more neoplastic, there is a switch to the ovarian-type promoter which is inhibited by RXR agonists [Endocrinology 2002;143:2863–71]. So, I don't really have an answer for you, bar the fact that it was an unanticipated finding that the RXR agonist could inhibit the aromatase promoter in the indirect manner proposed. I don't think anyone knows about the use of different promoters as tumors progress.

Dr. Santen: The standard thinking is that increasing MAP kinase increases phosphorylation of serine 118 and 157, which results in increased transcription by the estrogen receptor. But it is equally plausible that MAP kinase is actually directly stimulating cell proliferation independently of the estrogen receptor under those circumstances. You were focusing on transcription, but one would have to basically take and make serine mutations of the estrogen receptor and then look at the SDF-1 to see if you could block proliferation when you increased MAP kinase under those circumstances.

Dr. McDonnell: I actually don't think that the proliferation is through the estrogen receptor. I think what happens is that estrogens induce SDF-1, which induces MAP kinase, and the MAP kinase does all the things it is supposed to do, but there is a bit of a shunt wherein MAP kinase's actions lead to an increase in ER transcriptional activity. Additionally, it turns out that one of the things MAP kinase does is to cause degradation of PPARγ. That may be the primary mechanism for shutdown of PPARγ signaling. So when you activate MAP kinase, not only do you put the accelerator on, with respect to the estrogen receptor, you take off the brake by decreasing PPARγ levels. So it is a little bit more difficult than just looking at the phosphorylation state of either receptor.

Dr. Santen: I raise that question because when Rob Nicholson talks about hormone resistance, he talks about MAP kinase activating the estrogen receptor through phosphorylation. The way to distinguish this is to mutate the estrogen receptor so that it can't be phosphorylated and then look to see whether inhibitors of MAP kinase will alter proliferation under those circumstances. I think it's really a key issue.

Dr. McDonnell: It is, but the issue has been addressed by Myles Brown in the paper that he published last year [Proc Natl Acad Sci U S A 2004;101:11599–604]. Even though the estrogen receptor can be phosphorylated at serine 118, his data suggests that was irrelevant. He basically said that it was phosphorylation of AIB1 that was the primary target.

Dr. Kent Osborne: In the HER2-overexpressing and the wild-type MCF-7 cells, we found that tamoxifen phosphorylates ER potently at serine 118 and yet it is a potent antiestrogen in the MCF-7 cells. That result just said to us that phosphorylation of the ER is not by itself an important factor for resistance.

Dr. McDonnell: I think there was a tendency that we all had in the field to be reductionists, to think that it was going to be so simple, that everything was going to converge on one amino acid. Myles Brown's paper basically showed that AIB1 phosphorylation was the primary target within the ER signal transduction pathway that is targeted by MAPK.

Dr. Santen: But that still doesn't address the direct effect of MAP kinase on proliferation independently of or in addition to the effect of MAP kinase to enhance transcriptional regulation either by the coactivator or by serine 157, which is the other target, or via other phosphorylation mechanisms.

Dr. McDonnell: There are two hypotheses here. One is that activation of MAP kinase stimulates proliferation and the receptor is downstream of that. I don't subscribe to that hypothesis. I subscribe to the hypothesis that MAP kinase activates other processes downstream that allow it to activate transcription and they are independent of the estrogen receptor. I would suggest, obviously without a lot of data, that the mechanism by which estrogens participate in proliferation is by activating CXCR4 in an indirect manner and then the activated MAP kinase modulates the ability of the estrogen receptor to function as a transcription factor. There are also effects on proliferation mediated by MAP kinase that occur independently of ER.

Dr. Santen: Our own studies are really suggesting that it is the PI3K pathway that is involved in proliferation, particularly mTOR much more than MAP kinase. So I would ask whether the mTOR inhibitors rapamycin or CCI779 would block the SDF-1 stimulation.

Dr. McDonnell: We've marched down all of the pathways now and downstream of CXCR4—CXCR4 activates Gq, which then activates MAP kinase, but Gq can go to PI3K as well. So it is quite possible that both pathways are activated at the same time and that basically if you inhibit one or the other that you are going to get an effect. It is like a three-legged stool; take one leg away and the whole thing falls over, so it doesn't make any difference which one you inhibit.

Dr. Angela Brodie: There might be a gradation in what is actually occurring as cells switch to these pathways starting with

estrogen deprivation. As you increasingly pressure the cells to make them more and more deprived, they go from estrogen-dependent pathways down into the independent pathways and MAP kinase. We have some evidence for that.

Dr. McDonnell: That is very likely and, given your data, that would seem to be the way it is going.

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