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Newly-Recognized Small Molecule Receptors on Human Breast Cancer Cell Integrin αvβ3 that Affect Tumor Cell Behavior

Hung-Yun Lin[1], Faith B. Davis[1], Mary K. Luidens[1,2], Aleck Hercbergs[3], Shaker A. Mousa[4], Dhruba J. Bharali[4] and Paul J. Davis[1,2]
[1]*Signal Transduction Laboratory, Ordway Research Institute, Albany, NY,*
[2]*Albany Medical College, Albany, NY,*
[3]*Cleveland Clinic, Cleveland, OH,*
[4]*Pharmaceutical Research Institute,*
Albany College of Pharmacy, Albany, NY,
USA

1. Introduction

Hormonal regulation of the growth of breast cancer cells has been largely seen to result from interactions of estrogen and progestins with nuclear receptors for these steroids that may reside, unliganded, in cytoplasm or be transcriptionally active as steroid-protein nuclear receptor complexes. Receptors for nonpeptide hormones exist in the plasma membrane, however, and when activated may stimulate breast cancer cell proliferation. For example, the functional classical estrogen receptor-α (ERα) is found in the cell membrane. Recently, the identification has been made of a novel receptor for thyroid hormone and for dihydrotestosterone (DHT) on the plasma membrane of cells; this receptor is the αvβ3 integrin, which promotes proliferation of human breast cancer cells by these two hormones. Integrins are heterodimeric structural proteins of the plasma membrane whose primary functions are to interact with extracellular matrix proteins and growth factors. One integrin, αvβ3, bears discrete receptors for thyroid hormone (L-thyroxine, T4; 3, 5, 3′-triiodo-L-thyronine, T3) and for DHT. A receptor for the polyalcohol, resveratrol, also exists on this integrin in breast cancer cells, mediating the anti-proliferative, pro-apoptotic action of this compound. Resveratrol has certain structural features that are estrogen-like. Disparate actions of T4, T3, DHT and resveratrol that are initiated at the integrin depend downstream upon stimulation of the activity of mitogen-activated protein kinase (MAPK), suggesting the existence of distinct, function-specific pools of MAPK within the cell. Tetraiodothyroacetic acid (tetrac) is a model specific inhibitor of hormone actions on the thyroid hormone integrin receptor. Tetrac and a nanoparticulate formulation of tetrac block stimulation by thyroid hormone analogues of cancer cell proliferation and of angiogenesis. Interestingly, tetrac also acts in the absence of T4 and T3 to block the tumor-relevant angiogenic responses to vascular growth factors, e.g., vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and other growth factors. Tetrac and nanoparticulate tetrac also

disable expressions of families of genes important to cancer cell survival pathways. This chapter reviews the functions of the several nonpeptide hormone receptors on integrin αvβ3.

The possibility that the hormone-directed biology of the breast cancer cell might be in part regulated from the cell surface was first suggested by the identification of estrogen receptor protein in the plasma membrane (Levin, 1999). Discrete receptors on the plasma membrane αvβ3 integrin of breast cancer cells have also recently been described for thyroid hormone (Bergh *et al.*, 2005; Cheng *et al.*, 2010; Davis, P. *et al.*, 2011), for dihydrotestosterone (DHT) (Lin, H. *et al.*, 2009b) and for resveratrol (Lin, H. *et al.*, 2006). The several functions of these membrane receptors include modulation of cancer cell proliferation and, in the case of thyroid hormone, of tumor-relevant angiogenesis (Cheng *et al.*, 2010; Mousa *et al.*, 2009). Expression of integrin αvβ3 is concentrated in tumor cells and rapidly-dividing endothelial and vascular smooth muscle cells, so that receptors for these hormones—and resveratrol is a polyalcohol with estrogen-like structural features—may be considered targets for manipulation of breast cancer. In the present review we will describe the features of these receptors in the breast cancer cell, and will also propose clinical therapeutic applications that are based on inhibition of these small molecule plasma membrane receptors.

2. Thyroid hormone stimulates human breast cancer cell proliferation via plasma membraneIntegrin αvβ3

In the absence of estrogen, thyroid hormone (L-thyroxine [T_4]) was shown in 2004 to enhance proliferation of human estrogen receptor-α (ERα)-positive breast cancer cells (Tang *et al.*, 2004). The thyroid hormone effect required extracellular-regulated kinases 1 and 2 (ERK1/2)-dependent phosphorylation of Ser-118 of ERα, precisely mimicking the action of estradiol (Kato *et al.*, 1995) on breast cancer cell proliferation. The nuclear estrogen receptor inhibitor ICI 182,780 (Fulvestrant®) blocked this action of thyroid hormone (Lin H. , unpublished), as did tetraiodothyroacetic acid (tetrac), an analogue of T_4 in which the alanine side chain of T_4 is converted to acetic acid (Tang *et al.*, 2004). Tetrac is an inhibitor of actions of T_4 and T_3 that are initiated at the thyroid hormone receptor site on integrin αvβ3 (Bergh *et al.*, 2005; Yalcin *et al.*, 2010a; Yalcin *et al.*, 2010b; Davis, F. *et al.*, 2006). Studied *in vitro*, T_4 and T_3 are anti-apoptotic in breast cancer cells (Tang *et al.*, 2004) and other tumor cells (Lin *et al.*, 2008a), at least in part via a mechanism that blocks effectiveness of the oncogene suppressor protein and pro-apoptotic factor, p53 (Lin *et al.*, 2011).

Acting via the plasma membrane integrin receptor, agonist thyroid hormone analogues T_4 and T_3 are also pro-angiogenic (Mousa *et al.*, 2009; Davis, P. *et al.*, 2009; Luidens *et al.*, 2010). In the absence of tumor cells and in the setting of tissue ischemia, stimulation of neovascularization by T_4 and T_3 may be desirable (Tomanek *et al.*, 1998; Chen *et al.*, 2010). In the setting of cancer, however, these agents appear to enhance tumor-related angiogenesis. The mechanism of angiogenesis is complex and involves the release by endothelial cells of vascular growth factors (Tomanek *et al.*, 1998) and the resulting autocrine effects of such factors. T_4 and T_3 may also enhance the actions of vascular growth factors (Davis, F. *et al.*, 2004).

The effectiveness of tetrac as an inhibitor of actions of T_4 and T_3 on the plasma membrane of cancer cells caused us to study tetrac as an anti-cancer and anti-angiogenic agent. We found

that unmodified tetrac as well as tetrac re-formulated as a nanoparticulate, in which it is covalently bound to poly-lactic-co-glycolic acid (PLGA), have anti-proliferative effects in tumor cells. These actions reflect the ability of tetrac and nanoparticulate tetrac to 1) antagonize the pro-proliferative, anti-apoptotic actions of T_4 and T_3; 2) to disable, in the absence or presence of T_4 and T_3, the expression of a number of survival pathway genes (Glinsky *et al.*, 2004); and 3) to *suppress* the *death-from-cancer* gene signature of a number of cancer cell lines (Glinsky *et al.*, 2005). This 11-gene signature is a predictor of aggressiveness of cancer cells as demonstrated by shortened "time-to-tumor recurrence", the presence of distant metastasis, and death after tumor therapy. The PLGA formulation of tetrac acts exclusively at the integrin receptor for thyroid hormone; in contrast, unmodified tetrac acts at the integrin, but also gains access to the interior of the cell and may mimic actions of T_4 (Moreno *et al.*, 2008).

Actions of nanoparticulate tetrac on gene expression in human breast cancer cells are coherent (Glinskii *et al.*, 2009). While the expression of a number of cyclin genes is suppressed and *anti*-apoptotic gene expression is decreased, *pro*-apoptotic gene expression is enhanced. Fairly remarkably, the expression of *thrombospondin 1* (*TBSP1*) is increased. The TBSP1 protein is anti-angiogenic and the protein rarely accumulates in cancer cells. Nanoparticulate tetrac can also suppress expression of the *epidermal growth factor receptor* (*EGFR*) whose gene product supports cancer growth and angiogenesis and whose receptor is an oncologic target (Glinskii *et al.*, 2009). In the same study, however, while unmodified tetrac blocked expression of cyclin genes and certain genes relevant to apoptosis, this unmodified form of tetrac did *not* affect *EGFR* expression. Thus, the fit of the nanoparticulate formulation into its receptor on integrin αvβ3 appears to be distinct from that of tetrac, itself. Nanoparticulate tetrac is also 10- to 100-fold more potent than unmodified tetrac, depending on the particular cell line studied (Glinskii *et al.*, 2009; Yalcin *et al.*, 2010a; Yalcin *et al.*, 2010b).

These observations on the two formulations of tetrac are consistent with the complexity of the receptor for thyroid hormone on the integrin (Cody *et al.*, 2007) and with the ability of the integrin to generate a spectrum of intracellular actions via several signal-transducing kinase pathways, including MAPK (extracellular-regulated kinases 1/2, or ERK1/2) and phosphatidyl-inositol 3-kinase (PI3K). Studied in human glioma cells by mathematical modeling of the kinetics of binding, the receptor site appears to contain two thyroid hormone-binding domains, one that recognizes only T_3 and a second that transduces both the T_4 and T_3 signals (Lin *et al.*, 2009c). Recent computer graphics analysis of the interactions of T_3 and T_4 with the integrin (V. Cody, unpublished observations) is consistent with the existence of two hormone-binding domains in the receptor. While tetrac and nanotetrac interact with both domains, they do so differently, as the resulting gene expression profiles obtained in breast cancer cells indicate (Glinskii *et al.*, 2009).

It is important to note that the integrin-mediated anti-angiogenic activity of tetrac and nanotetrac transcend the inhibition of the pro-angiogenic thyroid hormone agonists, T_4 and T_3. That is, in the absence of agonist thyroid hormone, tetrac can block the actions of vascular endothelial growth factor (VEGF) (Mousa *et al.* , 2008), bFGF (FGF2) (Mousa *et al.* , 2008), platelet-derived growth factor (PDGF) (SA Mousa: unpublished observations) and, as mentioned above, EGF. Because tumor cells can secrete multiple vascular growth factors to support their needs, it is desirable to identify potential therapeutic agents that antagonize the actions of more than one such growth factor.

It is clear from the foregoing that the actions of tetrac formulations at the cell surface integrin and of T_4 and T_3 at αvβ3 generate complex downstream events. However, it is also apparent that the hormone receptor on the integrin can also engage in crosstalk with growth factors on the plasma membrane. Interference by tetrac formulations with the actions of VEGF, bFGF and other growth factors imply this, since such growth factors include Arg-Gly-Asp (RGD) sequences that are validated by the RGD recognition site on the integrin before each growth factor can activate its growth factor-specific receptor clustered with the integrin (Davis, P. *et al.*, 2011). The αvβ3-vascular growth factor receptor interaction, e.g., with VEGF (Sarmishtha *et al.*, 2005) and bFGF (Sahni and Francis, 2004), have been described in other studies that did not include thyroid hormone. The integrin receptor for agonist thyroid hormone analogues and for tetrac, however, influences other local cell membrane events. For example, activity of the Na^+/H^+ exchanger (NHE1) is regulated by thyroid hormone analogues and this action is blocked by tetrac (Incerpi *et al.*, 2003). The insertion of Na, K-ATPase protein into the plasma membrane and activity of the ATPase (sodium pump) are also affected nongenomically by a MAPK- and PI3K-dependent mechanism (Lei *et al.*, 2007).

Such observations caused us to examine in doxorubicin-resistant human breast cancer cells (MCF-7) whether the state of resistance, in part determined by the expression and activity of the P-glycoprotein (MDR) pump (Dönmez *et al.*, 2010), may be affected by tetrac. Treatment of such breast cancer cells with tetrac reversed chemoresistance of the cells and resulted in increased intracellular residence time of radiolabeled doxorubicin (Rebbaa *et* al., 2008). Such i*n vitro* studies suggest that the combination of tetrac and doxorubicin should be tested in xenografts of doxorubicin-resistant MCF-7 cells. The mechanism of this particular action of tetrac has not been established. While it is possible that the function of the P-glycoprotein is affected by crosstalk with the tetrac-occupied hormone receptor on the integrin, we have also suggested that the action of tetrac on the NHE may be involved (Incerpi *et al.*, 2003). That is, inhibition of the NHE and a resultant intracellular decrease in pH may affect P-glycoprotein function because of the pH optimum of the MDR pump.

We would also point out that tetrac radiosensitizes tumor cells (Hercbergs *et al.*, 2009) by interfering with repair of DNA double-strand breaks (Hercbergs *et al.*, 2011). However, this has been studied to-date only in murine and human glioma cells and whether the agent affects DNA repair in breast cancer cells is not yet known.

A final consideration with regard to thyroid hormone is the recently described effect of the hormone on the abundance of integrin αvβ3 on the tumor cell plasma membrane. Agonist thyroid hormone analogues have a modest effect on the expression of the αvβ3 gene (Yonkers *et al.*, 2009) and may also increase the internalization of the αv, but not the β3 component (Lin *et al.*, 2009). The disparate distribution of the αv and β3 monomers within the cell is also conditioned by thyroid hormone, including import of αv into the cell nucleus (Lin *et al.*, 2007).

3. Actions of resveratrol and the integrin receptor on the biology of breast cancer cells

Resveratrol is an extensively-studied, naturally-occurring alcohol with desirable properties in several biologic models. These activities include extension of lifespan in *C. elegans* (Zarse *et al.*, 2010) and remarkable anti-cancer properties (Pezzuto *et al.*, 2011; Hsieh *et al.*, 2011; Lin, C *et al.*, 2011). Substantial attention has been devoted to the metabolism of this agent because of its

rapid disappearance from the circulation post-administration to intact animals and its cellular uptake and chemical modification (Delmas *et al.*, 2011). The half-life of the parent compound is sufficiently short to promote speculation about the nature of the active biologic material.

We recently described a cell surface receptor for resveratrol on breast cancer cells which, like the thyroid hormone receptor on tumor cells (Bergh *et al.*, 2005), is on integrin αvβ3 (Lin *et al.*, 2006). The existence of such a receptor and its ability to transduce the plasma membrane resveratrol signal into MAPK activity and downstream into pro-apoptotic action suggested that the parent compound has substantial bioactivity. Integrins have been widely-viewed to bear receptors or binding sites only for large molecules—extracellular matrix proteins and growth factors (Plow *et al.*, 2000)—and thus it was surprising to find apparent biologically relevant binding sites for two small molecules on this integrin. It was also remarkable that the receptors for thyroid hormone and for resveratrol did not appear to interact functionally with one another. That is, both agents activated intracellular pools of MAPK (ERK1/2), but resveratrol was pro-apoptotic (Lin H *et al.*, 2008a; Lin C *et al.*, 2011) whereas thyroid hormones (T_4, T_3) were anti-apoptotic (Lin H *et al.*, 2008b), as noted above. Such observations suggested that the results of any efficacy testing of resveratrol as a chemotherapeutic agent in the presence of physiologic concentrations of thyroid hormone *in vitro* or in the intact animal with a normal pituitary-thyroid axis may be difficult to interpret (see below). An Arg-Gly-Asp (RGD) peptide prevents both the pro-apoptotic activity of resveratrol and the anti-apoptotic activity of thyroid hormone, indicating that the receptor is near the RGD recognition site on the integrin that was mentioned above (see section on *Thyroid Hormone Action*). The interactions of tetrac with either T_4 or resveratrol, however, indicate that the two integrin binding sites are distinct, in that while tetrac inhibits the proliferative action of T_4 in cancer cells, tetrac does not inhibit the pro-apoptotic actions of resveratrol (Lin C *et al.*, 2011).

Our own studies of the mechanism by which resveratrol may act to induce apoptosis in tumor cells have revealed several unexpected findings. First, resveratrol is able to induce p53-related apoptosis in cancer cells expressing certain mutations in p53. What is required in p53 for expression of resveratrol's apoptotic activity is that the Ser-15 be intact, as it is a target of phosphorylation by resveratrol-activated MAPK (Lin H *et al.*, 2002). Second, one of the actions of resveratrol in tumor cells is to induce a nuclear pool of cyclooxygenase-2 (COX-2) (Lin C *et al.*, 2011; Lin H *et al.*, 2009a). Chronic accumulation of COX-2 in cytoplasm is a marker of tumor cell aggressiveness (Schmitz *et al.*, 2006; Perdiki *et al.*, 2007) and long-term pharmacologic inhibition of the enzymatic activity of COX-2—the product of which is prostaglandins—appears to improve clinical outcomes or prevent emergence of certain cancers, such as that of the colon (Galalmb *et al.*, 2010).

Inducible COX-2 in the nucleus, however, is a wholly different biologic product. It is pro-apoptotic, can interact with Ser-15-phosphorylated p53 and can even bind to DNA (Lin H *et al.*, 2008b). The latter observation raises the possibility that COX-2 can be a co-activator and, indeed, resveratrol-induced nuclear COX-2 co-localizes with p300, a coactivator for p53 (Song *et al.*, 2010), as well as for proteins in the superfamily of nuclear hormone receptors (Kalkhoven, 2004). Activation of MAPK is required for formation of the complex of p53, p300 and COX-2, since that complex is not obtained in resveratrol-treated cells in the presence of the MEK-MAPK inhibitor, PD 98059 (Tang *et al.*, 2006). In contrast, inhibition of the enzymatic activity of COX-2 with indomethacin does not affect the pro-apoptotic activity of COX-2 (Tang *et al.*, 2006).

Third, a thyroid hormone analogue such as T_4 prevents or disrupts the formation of the nuclear p53-COX-2 complex in cells treated with resveratrol (Lin *et al.*, 2008a, b). It is thought that this hormonal effect explains the blunting of the pro-apoptotic action of resveratrol in the presence of thyroid hormone. Competition between thyroid hormone and resveratrol for binding to the αvβ3 integrin is thought to be responsible for this inhibition. Not unexpectedly, in a tumor cell system that includes resveratrol and a physiologic concentration of T_4, the addition of tetrac protects the pro-apoptotic action of resveratrol from the anti-apoptotic effect of thyroid hormone (Lin *et al.*, 2008b). All of these actions compete at the αvβ3 integrin receptor. In the absence of T_4, the induction of apoptosis by resveratrol is not affected by the addition of tetrac (Lin *et al.*, 2008b).

The antecedent review suggests that further testing of resveratrol as a cancer chemotherapeutic agent might be pursued in two ways. First, the combination of nanotetrac and resveratrol may be evaluated against breast tumor cells *in vitro* or *in vivo* in the nude mouse xenograft model. Second, the potential manufacture of a nanoparticulate formulation of resveratrol in which the polyalcohol is covalently bound to the nanoparticle may be desirable, so as to permit biologic activity but restrict that effect of resveratrol to the integrin receptor. This formulation would prevent cellular uptake and subsequent metabolism/degradation of the nanoparticulate compound.

There is another implication with regard to the dependence of the pro-apoptotic action of resveratrol on an inducible pool of nuclear COX-2. New pharmacologic inhibitors of the enzymatic function of cyclooxygenase-2 should be examined for their ability to block the action of resveratrol, and perhaps of other polyalcohols as model inducers of nuclear COX-2. For example, NS398 is an experimental inhibitor of COX-2 that has been shown to prevent induction of COX-2 by resveratrol (Tang *et al.*, 2006).

4. Dihydrotestosterone (DHT) acts via integrin αvβ3 to induce proliferation of human breast cancer cells

Although androgens may have an inhibitory effect on the proliferation of breast cancer cells, the actions of these steroids on such cells are variable. When we examined the action of dihydrotestosterone (DHT) on ERα-positive MCF-7 and ERα-negative MDA-MB-231 human breast cancer cells, we found that the androgen promoted proliferation in both cell lines (Lin *et al.*, 2009b). Integrin αvβ3 antibody inhibited the action of DHT in MDA-MB-231 cells, but was ineffective in MCF-7 cells (Lin *et al.*, 2009b). On the other hand, ICI 182,780 treatment and siRNA knockdown of ERα blocked the proliferative effect of DHT in MCF-7 cells, but not in the MDA-MB-231 cells. Thus, the mechanisms of DHT action differ in the two cell lines, and only in the ER-negative cells was there evidence for the existence of a DHT receptor on integrin αvβ3. In neither breast cancer cell line could participation of a classical androgen receptor be implicated in the action of DHT. Tetrac did not affect the action of DHT (HY Lin: unpublished observations), indicating that these two small molecule receptors on the integrin function independently of one another.

It is not yet clear what the clinical significance may be of the DHT receptor on the breast cancer cell surface. We speculate, however, that in the patient with a recurrent ERα-positive tumor and taking tamoxifen or an aromatase inhibitor, residual circulating androgen may be promoting cancer cell proliferation. Useful information relevant to this possibility will come from determination of the androgen analogue-specificity of the receptor for DHT, and of a

possible contribution of DHT to breast cancer cell growth. In the case of the ER-negative human breast cancer cell, we have already demonstrated its susceptibility to DHT-stimulation (Lin *et al.*, 2009b).

It will also be important to analyze solid tumors beyond breast cancer for the presence of DHT-induced growth stimulation, including cancer cell growth as well as angiogenesis. In a recent report, Sieveking *et al.* (2010) have reported that androgen treatment of male endothelial cells *in vitro* enhanced angiogenesis; in contrast, gene knockdown of the androgen receptor (AR) in these cells caused unresponsiveness of these cells to androgen treatment. Female endothelial cells lacking AR did not respond to androgen treatment, but overexpression of the androgen receptor in female endothelial cells caused angiogenesis to occur.

5. Discussion and conclusions

Modulation of the proliferation of breast cancer cells is largely viewed as a function of the presence or absence of estrogen in ER-positive tumor cells and the presence of polypeptide growth factors that are autocrine or systemic. The emphasis on ER-mediated actions has grown from a broad understanding, emerging from a number of laboratories, of the molecular functions of ER in the cell nucleus and genomic actions of estrogens. Management of breast cancer—beyond surgery, tumor irradiation and chemotherapeutic agents—specifically emphasizes suppression of the action of endogenous estrogen with tamoxifen or inhibition of estrogen synthesis with aromatase inhibition. That estrogen may support breast tumor growth nongenomically is now under consideration (Silva & Shupnik, 2007), perhaps involving nuclear ERα insinuated into the plasma membrane or ER-like proteins in the membrane (Levin, 2011).

The concept that thyroid hormone may be a growth factor for breast cancer has been advanced by various investigators (Goodman *et al.*, 1980; Borek *et al.*, 1983; Cristofanilli *et al.*, 2005). That thyroid hormone could nongenomically support ER-positive human breast cancer cell proliferation and to be estrogen-like was shown when T_4 caused MAPK-dependent specific serine phosphorylation of nuclear ER, mimicking estradiol upon which depended stimulation of proliferation by the iodothyronine. This work led to the subsequent identification of a thyroid hormone receptor on plasma membrane integrin αvβ3 at which a variety of effects of thyroid hormone are initiated that are nongenomic in mechanism (Tang *et al.*, 2004). The integrin (and, therefore, the receptor) is expressed primarily on cancer cells of various types and on rapidly-dividing blood vessel cells (Dijkgraaf *et al.*, 2009; Wei *et al.*,2009; Dimastromatteo *et al.*, 2010).

An interesting inhibitor of this receptor target exists. This is tetraiodothyroacetic acid (tetrac), a deaminated analogue of T_4 that blocks binding of T_4 and T_3 to the integrin, but also has novel anti-cancer activities at the αvβ3 hormone-binding site in the absence of T_4 and T_3. These actions, initiated at the cell surface, are on expression of specific genes and the actions are inimical to tumor cell survival and on angiogenesis (Glinskii, A. *et al.*, 2009), including that initiated by several vascular growth factors. The hormone receptor on the integrin also disables or garbles crosstalk between the integrin and growth factor receptors that are clustered with αvβ3 (Davis, F. *et al.*, 2004; Mousa S. *et al.*, 2008).

Tetrac has been re-formulated as a nanoparticle in which the outer ring hydroxyl group is stably covalently bonded through a linker to the nanoparticle. This formulation does not

enter the cell and its actions are limited to the tetrac/thyroid hormone receptor on integrin αvβ3 (Bergh J. *et al.*, 2005). The agent has been shown to suppress growth of a variety of human cancer cell xenografts and to be anti-angiogenic at the tumor site (Davis F. *et al.*, 2004; Davis F. *et al.*, 2006; Glinskii A. *et al.*, 2009; Davis P. *et al.*, 2011). This hormone target on the integrin is fairly remarkable, in that as a single target, it has a relatively large number of downstream actions on cancer cells that emphasize vulnerability of such cells.

Recognition and characterization of the thyroid hormone receptor on integrin αvβ3 were premonitory to the identification of other small molecule binding sites—now understood to be receptors in that, when occupied, they cause predictable downstream cellular events. These receptors appear to be quite independent of one another and of the thyroid hormone binding domain. Identification of the resveratrol receptor on the integrin of breast cancer cells and other solid tumor cells may provide useful insights into the actions of this stilbene. The rapid cellular uptake and metabolism of resveratrol (Kroon *et al.*, 2010)—leading to pharmacokinetic and pharmacodynamic speculation about the relative bioactivity of resveratrol analogues—is less puzzling if, in fact, the parent compound (resveratrol) can act on the outside of the cell. That is, resveratrol binds to the integrin and rapidly initiates a MAPK-requiring, p53-dependent apoptotic process, regardless of uptake of the compound and chemical processing. What was somewhat surprising was the observation that resveratrol-induced apoptosis was blocked by thyroid hormone, but by a mechanism that begins at a different domain on the integrin and utilizes MAPK, but is effected by interruption of a resveratrol-induced phosphorylation sequence in the cancer cell nucleus that involves p53 and other nucleoproteins. The outcomes of a recent pharmaceutical industry-sponsored clinical trial of resveratrol as an anti-cancer agent were disappointing (McBride, 2010). Where there may be multiple explanations for these results, we can speculate that in the setting of normal thyroid function that the anti-cancer activity of resveratrol may be wholly suppressed by the hormone.

A third small molecule receptor on the integrin of breast cancer cells was recently described and this is a site that responds to the androgen, dihydrotestosterone (DHT) (Lin, H. *et al.*, 2009b). It was not wholly unexpected that an authentic steroid was found to act at the integrin, since resveratrol, while not a steroid, has certain structural features and functional activities that are estrogen-like. Acting via integrin αvβ3, DHT is a trophic agent for breast cancer cells. Of some interest is that the mechanisms by which DHT acts *in vitro* differ in ERα-negative and ERα-positive cells. The cell surface integrin receptor for DHT is required for the proliferative effect of the androgen in ERα-negative cells, but is irrelevant in ER-positive cells, where the estrogen receptor protein, itself, is needed for DHT action. In neither type of cell is the authentic nuclear androgen receptor a part of the mechanism by which DHT recognizes the existence of a receptor on integrin αvβ3 in ERα-negative breast cancer cells. As we have pointed out above, recognition of the existence of the androgen receptor on αvβ3 in ERα-negative breast cancer may help to explain recurrences of tumor in postmenopausal women in whom some effect of circulating androgen, now apparent in the absence of estrogen, may be seen.

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7. References

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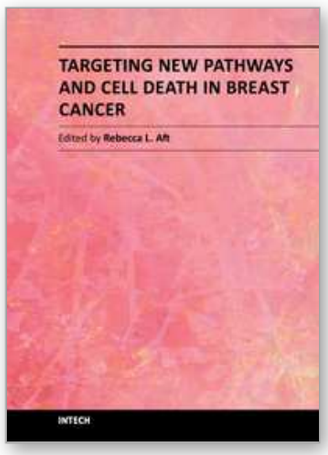

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