Review Article

Thyroid Hormone and P-Glycoprotein in Tumor Cells

Paul J. Davis, Sandra Incerpi, Hung-Yun Lin, Heng-Yuan Tang, Thangirala Sudha, and Shaker A. Mousa

1 Department of Medicine, Albany Medical College, Albany, NY 12208, USA
2 Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, 1 Discovery Drive, Rensselaer, NY 12144, USA
3 Department of Sciences, University Roma Tre, 00146 Rome, Italy
4 PhD Program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei 110, Taiwan

Correspondence should be addressed to Paul J. Davis; pdavis.ordwayst@gmail.com

Received 5 July 2014; Accepted 4 September 2014

Academic Editor: Yoshinori Marunaka

Copyright © 2015 Paul J. Davis et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

P-glycoprotein (P-gp; multidrug resistance protein 1, MDR1; ABCB1) is a plasma membrane efflux pump that when activated in cancer cells exports chemotherapeutic agents. Transcription of the P-gp gene (MDR1) and activity of the P-gp protein are known to be affected by thyroid hormone. A cell surface receptor for thyroid hormone on integrin \( \alpha v \beta 3 \) also binds tetraiodothyroacetic acid (tetrac), a derivative of L-thyroxine (T\(_4\)) that blocks nongenomic actions of T\(_4\) and of 3,5,3' -triiodo-L-thyronine (T\(_3\)) at \( \alpha v \beta 3 \). Covalently bound to a nanoparticle, tetrac as nanotetrac acts at the integrin to increase intracellular residence time of chemotherapeutic agents such as doxorubicin and etoposide that are substrates of P-gp. This action chemosensitizes cancer cells. In this review, we examine possible molecular mechanisms for the inhibitory effect of nanotetrac on P-gp activity. Mechanisms for consideration include cancer cell acidification via action of tetrac/nanotetrac on the Na\(^+\)/H\(^+\) exchanger (NHE1) and hormone analogue effects on calmodulin-dependent processes and on interactions of P-gp with epidermal growth factor (EGF) and osteopontin (OPN), apparently via \( \alpha v \beta 3 \). Intracellular acidification and decreased H\(^+\) efflux induced by tetrac/nanotetrac via NHE1 is the most attractive explanation for the actions on P-gp and consequent increase in cancer cell retention of chemotherapeutic agent-ligands of MDR1 protein.

1. Introduction

P-glycoprotein (P-gp; multidrug resistance protein 1, MDR1; ABCB1) is a plasma membrane efflux pump with broad ligand specificity in normal cells and in cancer cells [1]. A glycoprotein ATPase is responsible in cancer cells for the outward transport of a variety of chemotherapeutic agents and thus is a critical vehicle of chemoresistance. P-gp is subject to pharmacologic inhibition with a variety of agents, for example, the calcium channel blocker, verapamil [1, 2], and tyrosine kinase inhibitors [3]. The search for effective P-gp inhibitor drugs is active [4, 5]. Thyroid hormone, L-thyroxine (T\(_4\)) or 3,3',5'-triiodo-L-thyronine (T\(_3\)), is known to induce transcription of P-glycoprotein (MDR1) gene [6–8] and P-gp function [8]. We have shown that a thyroid hormone antagonist, tetraiodothyroacetic acid (tetrac), acting at the thyroid hormone-tetrac receptor on plasma membrane integrin \( \alpha v \beta 3 \), increases the intracellular residence time of doxorubicin in chemoresistant (doxorubicin-resistant) human breast cancer cells [9]. This is an index of inhibition of P-gp activity. Thus, in cancer cells, this function of the hormone supports drug resistance, whereas in nonmalignant cells, this action of the hormone may stimulate desirable efflux of toxic substances accumulated by the cells. In this review, we examine the mechanisms by which thyroid hormone, tetrac and nanoparticulate tetrac formulation (nanotetrac) that acts exclusively at integrin \( \alpha v \beta 3 \), may regulate P-gp function in cancer cells. The integrin is also known to regulate P-gp by other mechanisms [10].

2. Integrin \( \alpha v \beta 3 \) and Nongenomic Actions of Thyroid Hormone

Integrins are structural proteins of the plasma membrane that bind extracellular matrix (ECM) proteins and are integral to
cell-cell adhesion and cell-ECM protein interactions. Among ECM protein ligands of various integrins are fibronectin, vitronectin, osteopontin (OPN), and von Willebrand factor [11]. Of more than 20 integrins, only αvβ3 contains a receptor site for thyroid hormone [12]. αvβ3 is amply expressed by tumor cells and rapidly dividing endothelial cells usually found supporting cancers. We have described cancer cell proliferation in vitro in response to T4 and T3 in a variety of human cells [13–15] and these hormones are proangiogenic by a variety of mechanisms [16–18]. Both actions are wholly dependent upon the hormone-tetra-Receptor on integrin αvβ3. Such actions of T4 and T3 at the integrin are termed nongenomic because they do not primarily require the interaction of nuclear thyroid hormone receptors (TRs) with T3, the definition of the genomic mechanism of hormone action [19]. T4 is active at the integrin and the affinity of the hormone receptor on αvβ3 is higher for T4 than for T3; in contrast, T3 in genomic actions is a prohormonal source of T3 via deiodination.

Tetra and nanotetra inhibit binding of agonist thyroid hormone to the receptor on the ectodomain of αvβ3. But, in the absence of T4 and T3, nanotetra and tetrac have a set of nuclear proapoptotic and antiangiogenic actions [17]. These involve modulation of crosstalk between the integrin and adjacent vascular growth factor receptors, the promotion of apoptosis, and the disordering of transcription of genes important to cell survival pathways [17, 30]. Specifically, there is crosstalk between αvβ3 and receptors for vascular endothelial growth factor (VEGF) [17] and epidermal growth factor (EGF) [17] that may be relevant to the αvβ3-mediated effects of thyroid hormone on cellular retention of chemotherapeutic agents (see next section).

From the integrin, T4 can also alter intracellular trafficking and state of serine phosphorylation of TRs, of estrogen receptor-α (ERα), of signal transducing and activator of transcription (STAT) proteins, and of p53 [17]. These phosphorylation steps are dependent upon mitogen-activated protein kinase (MAPK; ERK1/2) and represent an interesting adjucative interface of nongenomic actions with genomic actions of thyroid hormone. In human lung carcinoma cells that express ERα, T4 may be estrogen-like, supporting cell proliferation that is ER-dependent [15]. Migration of endothelial cells toward a vitronectin cue is also stimulated by T4 via αvβ3 [18]. Fibroblast migration in an in vitro model of wound-healing is also stimulated by T4 at the cell surface hormone receptor (SA Mousa: unpublished observations). The state of the actin cytoskeleton is nongenomically regulated by T4 [31, 32], in part reflecting action of the hormone to increase the amount of fibrous (F) actin from the pool of available soluble actin.

Finally, thyroid hormone can nongenomically regulate the activities of several plasma membrane transport systems, including the sodium/proton (Na+/H+) exchanger (NHE1) or antiporter [33, 34], Na+, K+ -ATPase [35, 36], and the glucose transport system [37]. The action on NHE1 contributes to regulation of intracellular pH (pHi). Inhibition of this integrin-mediated effect of thyroid hormone decreases cellular pH and may permit modulation of activity of enzymes whose pH optima are physiologic or slightly alkaline. Increased activity of NHE1 will also decrease extracellular pH (pHe), an effect that may reduce cell uptake of certain chemotherapeutic agents [21]. The plasma membrane calcium pump (Ca2+-ATPase) is another ATPase whose transport activity is activated nongenomically by T4 [38–40].

3. Possible Mechanisms by Which Tetra and Agonist Thyroid Hormone Cause Tumor Cell Retention of Chemotherapeutic Agents

When we studied doxorubicin-resistant human breast cancer (MCF-7/dox) cells in vitro, we confirmed shortened intracellular residence time of labeled doxorubicin in these cells [9]. Tetrac exposure significantly increased residence time of doxorubicin in MCF-7/dox cells. The residence time of etoposide and cisplatin in neuroblastoma and osteosarcoma cell lines was also increased by tetrac. Of importance here is that doxorubicin and etoposide are P-gp substrates, whereas cisplatin is not. P-gp may influence the activities of certain apoptosis-relevant proteins such as p53 and caspase-3 and thus increase cancer cell sensitivity to agents such as cisplatin that are not P-gp substrates [41]. This indicates that tetrac may inactivate mechanisms of resistance in addition to the efflux pump. In studies we have carried out [9], we found that tetrac did not alter cellular abundance of superoxide dismutase (SOD) or glutathione-S-transferase-π (GST-π) proteins that support chemoresistance in the MCF-7/dox cell line. The P-gp protein abundance was ample in resistant cells but undetectable in wild-type MCF-7 cells. We postulated that tetrac decreased the activity of the P-gp ATPase to cause increased residence time of doxorubicin and etoposide, because agonist thyroid hormones (T4 and T3) nongenomically increase the activity of a variety of plasma membrane pumps—including several ATPases—and tetrac blocks nongenomic actions of T4 and T3, which are agonists at their receptor on αvβ3.

What are the molecular mechanisms that might be modulated by tetrac to result in decreased activity of P-gp and tumor cell retention of P-gp ligands such as doxorubicin and etoposide? Tetrac will block binding of thyroid hormone to integrin αvβ3 and if transcription of MDR1 is regulated from the cell surface, as is expression of a wide variety of genes [17, 30], then this action will decrease abundance of the protein in cancer cells. Thyroid hormone does increase transcription of MDR1 [6–8, 42]. This effect of the hormone does not involve the pregnane X receptor/steroid and xenobiotic receptor (PXR/SXR) [42] that is usually implicated in MDR1 gene expression, thus indicating the existence of one or more alternative pathways for regulation of MDR1 expression. Gene expression modulation from the integrin by thyroid hormone and tetrac formulations may involve alteration of the states of phosphorylation and acetylation of certain intranuclear receptors, as well as regulation of coactivator/corepressor complex formation [17]. Thus, it is not surprising that the hormone can affect MDR1 expression independently of PXR/SXR. Integrin αvβ3 has recently been shown to affect MDR1 expression by the phosphatidylinositol 3-kinase (PI3-K) /Akt pathway [10] that we have implicated in a variety of actions of thyroid hormone and tetrac initiated at this integrin [17].
Thyroid hormone also enhances function of the P-gp protein [8], but it is not yet known whether the latter effect is nongenomic in mechanism. Another possible mechanism of tetrac action on P-gp is sustained intracellular acidification, such as that induced pharmacologically with cariporide, an NHE1 inhibitor. This results in decreased P-gp activity [20] and also causes a reduction in MDRI (P-gp) gene expression and MDRI mRNA. Thyroid hormone acutely upregulates NHE1 activity and the inhibition of this nongenomic hormonal action by tetrac may result in a significant decrease in pH [33, 34], away from the pH optimum of the pump. In addition, a consequence of the tetrac effect on NHE1 is failure of the antiporter to support the extracellular acidosis that favors P-gp transport function [43, 44]. It is important to point out that the bovine serum-supplemented medium that cancer cells require for growth contains ample amounts of T4 and T3. We can conclude that one mechanism by which tetrac may downregulate activity of P-gp in tumor cells is via its αβ3-dependent action on NHE1. Recent reviews of P-gp chemistry and conceptual approaches to the inhibition of efflux pump activity have not considered acidification of P-gp-containing cells [1, 4, 5] as a strategy. This omission presumably reflects an assumption that pharmacologic acidification will affect normal cells, as well as tumor cells. This need not be the case when the pharmacologic initiation site is a protein such as integrin αβ3 whose expression/activation is primarily by tumor cells and rapidly dividing endothelial cells. A summary of molecular mechanisms by which tetrac and nanotetrac may affect P-gp function or abundance is presented in Figure 1.

As noted above, thyroid hormone action at αβ3 may also regulate activity of Na+-ATPase. A direct influence of change in [Na+]i, or [K+]i, on P-gp activity is not proposed, but inhibition by tetrac of the sodium pump will result in increased intracellular [Na+]i and decreased [K+]i. It is not known whether a specific change in intracellular [K+]i or [Na+]i affects P-gp, but inhibition by ouabain of Na-K-ATPase increases P-gp (MDRI) mRNA [45], suggesting that the monovalent cation microenvironment may directly or indirectly affect P-gp protein abundance. An indirect mechanism
Thyroid hormone is a regulator of [Ca\(^{2+}\)], via hormonal actions on plasma membrane Ca\(^{2+}\)-ATPase ("calcium pump") [39, 40]. This effect of thyroid hormone is dependent upon calmodulin. Verapamil has been shown by us to block the stimulatory effect of T\(_4\) on the calcium pump by interfering with the interaction of calmodulin with the ATPase [39]. Calmodulin is involved in control of P-gp activity through calmodulin-dependent kinase II activity [27, 28]. Thus, the conventional experimental use of verapamil to inhibit the P-gp axis may extend to calmodulin–relevant thyroid hormone actions that are linked to the efflux pump. It is not clear whether [Ca\(^{2+}\)], has roles in modulation of P-gp activity or the actions of tetrac/nanotetrac on the efflux pump, beyond generation of calmodulin–Ca\(^{2+}\) complexes.

A mechanism does exist by which agonist thyroid hormone (T\(_4\) or T\(_3\)) might decrease cell P-gp activity, as tetrac appears to do via αvβ3. The hormone induces cellular reactive oxygen species (ROS) generation [56, 57] and this may reduce P-gp [58, 59]. One of the coauthors of the present paper (S Incerpi) has shown that integrin αvβ3 is not involved in T\(_3\)-directed generation of ROS in hepatocytes [57]. Control thyroid hormone-containing (FBS-supplemented) culture medium for tumor cells does not increase intracellular residence time of chemotherapeutic agents [9] that is clearly seen with exposure of cells to tetrac.

4. Discussion

The observation that tetrac/nanotetrac can chemosensitize tumor cells previously resistant to agents such as doxorubicin and etoposide [9] caused us to undertake the present review of molecular mechanisms that may be the basis for actions of tetrac/nanotetrac on P-gp. Tetrac/nanotetrac oppose the nongenomic actions of T\(_4\) and T\(_3\) at plasma membrane integrin αvβ3 that regulate a variety of plasma membrane transport systems—such as the Na\(^+\)/H\(^+\) antiporter, Na, K-ATPase, and Ca\(^{2+}\)-ATPase [60]—that may be relevant to P-gp activity or to transcription of the MDRI (P-gp) gene. Further, integrin αvβ3 interacts with OPN and with the VEGF/VEGFR axis, offering opportunities for thyroid hormone analogues to modulate the influence of OPN and VEGF on P-gp. Table 1 summarizes a group of factors that modulate P-gp action and may be contributors to the increased intratumor intracellular residence time of chemotherapeutic agents in tetrac/nanotetrac–exposed tumor cells.

The most obvious molecular mechanism that contributes to the apparent effect(s) of tetrac/nanotetrac on P-gp is the action of these hormone analogues on intracellular pH. Tetrac acidifies cells by inhibiting the Na\(^+\)/H\(^+\) exchanger and the P-gp efflux pump is arrested by an acid intracellular environment. Here, the importance of the generous expression of the agent’s target—integrin αvβ3 with the tetrac receptor—on cancer cells is critical, so that conventional and necessary activity of MDRI in nonmalignant tissues is unimpaired in the presence of nanotetrac. Unmodified tetrac is unsatisfactory for cancer management because in the intact organism it is taken up by normal cells, as are T\(_4\) and T\(_3\). Within the normal cell, unmodified tetrac is a low-potency thyromimetic that can promote hypermetabolism.
A variety of additional pharmacologic inhibitors of P-gp are reviewed in [3–5]. NS: parameter not investigated/recorded. NC: no change in parameter. T4: L-thyroxine.

| Factor | P-gp activity | P-gp abundance | Reference |
|--------|--------------|----------------|-----------|
| Intracellular pH (pHi) | ↓ | ↓ | [20] |
| Extracellular pH (pHe) | ↑ | NS | [21] |
| Hypoxia | ↑ | ↑ | [22] |
| Hypoxia-inducible factor 1-α (HIF-1α) | NC | ↓ | [23] |
| Thyroid hormone/analogues | | | |
| T4, T3 | ↑ | ↑ | [6] |
| Tetrac/nanotetrac | ↓ | NC | [9] |
| Osteopontin (OPN) | ↓ | ↓ | [24] |
| Epidermal growth factor (EGF) | ↑ | NS | [25] |
| Vascular endothelial growth factor (VEGF) | ↓ | NC | [26] |
| Calcium channel blockers | ↓ | ↓ | [2] |
| Ouabain | NS | ↑ | [27] |
| Calmodulin antagonists E6, EBB | ↓ | NS | [28, 29] |

T4: L-thyroxine.
T3: 3,5,3'-triiodo-L-thyronine.
NC: no change in parameter.
NS: parameter not investigated/recorded.
A variety of additional pharmacologic inhibitors of P-gp are reviewed in [3–5].

The plasma membrane sodium pump and calcium pump are also regulated nongenomically by thyroid hormone. Inhibition of such nongenomic actions of thyroid hormone at αvβ3 by nanotetrac would serve to increase [Na+]i and [Ca2+]i. Such changes are not known to directly affect P-gp, although calmodulin–Ca2+ complexes are involved in calmodulin kinase-mediated effects that serve to increase P-gp activity, as mentioned above.

It is also apparent that P-gp and thyroid hormone analogues share mechanistic interests in a diverse set of protein molecules. As noted above, thyroid hormone increases transcription of the OPN gene and the OPN protein activates P-gp. Thus, in the clinical setting, host T3 (and T4 as a prohormone for T3) that acts via nuclear TR may support chemoresistance via P-gp. Nanotetrac is unlikely to affect P-gp via OPN because actions of nanotetrac are limited to αvβ3 and do not directly involve TR [17].

In contrast, EGF stimulates P-gp activity [25] and we have shown that, acting at the cell surface, thyroid hormone can potentiate certain effects of EGF [47]. Acting nongenomically, tetrac can inhibit agonist thyroid hormone action on EGF. Thus, a component of the prolongation of intracellular residence time of certain chemotherapeutic agents in nanotetrac-exposed cancer cells may be due to blockade of the action of T4 at the EGF receptor.

Recent reviews of regulation of P-gp [1, 4, 5, 61] endorse the search for new approaches to the efflux pump that are suitable for application to clinical chemoresistance. New approaches are facilitated by characterization of previously unrecognized control mechanisms for P-gp. We point out here that integrin αvβ3 offers access to multiple regulatory pathways for MDRI that may be suitable for pharmacological exploration. We have emphasized in this review the potential usefulness of the cell surface receptor on αvβ3 for thyroid hormone and tetrac/nanotetrac as a regulator of P-gp. However, the specific interactions of the integrin with extracellular matrix proteins, for example, OPN or growth factors, and existence on the integrin of other small molecule receptor sites offer new opportunities to modulate efflux pump activity.

Finally, it is interesting to note two additional interactions of thyroid hormone and P-gp. First, the export of the hormone from cells is a P-gp-mediated, verapamil-inhibitable process [62, 63] and thus to the extent that thyroid hormone may increase P-gp activity—or nanotetrac may inhibit such activity—intracellular hormone levels may be affected. For purposes of efflux, the hormone is a ligand of P-gp, but regulation by the hormone of P-gp activity is likely to originate at integrin αvβ3 and involve intermediary kinases implicated in transporter control [41]. Second, the extensive intracellular trafficking of P-gp among compartments is actin-dependent [64]. The integrity of the actin cytoskeleton and maintenance of F-actin is in part T4-regulated [31, 32]. The nongenomic actions of thyroid hormone on intracellular protein trafficking are reviewed elsewhere [17, 65].

In summary, P-glycoprotein (MDR1; ABCB1) is a ubiquitous plasma membrane efflux pump capable of exporting specific pharmacologic agents. In tumor cells, P-gp substrates include chemotherapeutic agents such as doxorubicin, etoposide, and trichostatin A. Thyroid hormone is known to stimulate expression of the MDR1 gene and activity of P-gp and thus may be seen to support chemoresistance. Tetrac is a thyroid hormone antagonist at the thyroid hormone–tetrac receptor on cell surface integrin αvβ3 and exposure of tumor cells to tetrac desirably increases retention time of the cancer chemotherapeutic agents that are known substrates of P-gp. A variety of molecular mechanisms are reviewed here by which thyroid hormone/tetrac may influence P-gp...
activity. Of interest is that cisplatin is not a substrate of P-gp, yet we have shown elsewhere [9] that its intracellular residence time is also increased by tetrac, raising the possibility of multiple mechanisms by which tetrac affects tumor cell handling of anticancer drugs. For example, tetrac may increase the activity of the organic cation transporter (OCT) [66] that imports (rather than exports) cisplatin, perhaps increasing the activity of the organic cation transporter (OCT) as discussed above. Thus, “intracellular residence time” of drugs in response to tetrac may reflect decreased P-gp efflux or, possibly, increased cationic transporter influx. The latter mechanism has not yet been examined.

Conflict of Interests

Coauthors Paul J. Davis and Shaker A. Mousa hold U.S. patents on nanoparticulate tetrac (nanotetrac). They receive no remuneration from ownership of these patents. The other coauthors have no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors appreciate the support of M. Frank and Marjorie D. Rudy for some of the studies described in this review.

References

[1] L. Zini, E. Capparelli, M. Cantore, M. Contino, M. Leopoldo, and N. A. Col tubo, “Small and innovative molecules as new strategy to revert MDR,” Frontiers in Oncology, vol. 4, Article ID Article 2, 2014.
[2] Y. Dönnmez, L. Akhmetova, Ö. D. Işeri, M. D. Kas, and U. Gündüz, “Effect of MDR modulators verapamil and promethazine on gene expression levels of MDRI and MRPI in doxorubicin-resistant MCF-7 cells,” Cancer Chemotherapy and Pharmacology, vol. 67, no. 4, pp. 823–828, 2011.
[3] L. N. Eadie, T. P. Hughes, and D. L. White, “Interaction of the efflux transporters ABCB1 and ABCG2 with imatinib, nilotinib, and dasatinib,” Clinical Pharmacology and Therapeutics, vol. 95, no. 3, pp. 294–306, 2014.
[4] Z. Binkhathlan and A. Lavasanifar, “P-glycoprotein inhibition as a therapeutic approach for overcoming multidrug resistance in cancer: current status and future perspectives,” Current Cancer Drug Targets, vol. 13, no. 3, pp. 326–346, 2013.
[5] R. Callaghan, F. Luk, and M. Bebawy, “Inhibition of the multidrug resistance P-glycoprotein: time for a change of strategy?” Drug Metabolism and Disposition, vol. 42, pp. 623–631, 2014.
[6] N. Nishio, T. Katsura, and K.-I. Inui, “Thyroid hormone regulates the expression and function of P-glycoprotein in Caco-2 cells,” Pharmaceutical Research, vol. 25, no. 5, pp. 1037–1042, 2008.
[7] K. Kurose, M. Saeki, M. Tohkin, and R. Hasegawa, “Thyroid hormone receptor mediates human MDRI gene expression—Identification of the response region essential for gene expression,” Archives of Biochemistry and Biophysics, vol. 474, no. 1, pp. 82–90, 2008.
[8] O. Burk, S. S. Brenner, U. Hofmann et al., “The impact of thyroid disease on the regulation, expression, and function of ABCB1 (MDRI/P glycoprotein) and consequences for the disposition of digoxin,” Clinical Pharmacology and Therapeutics, vol. 88, no. 5, pp. 685–694, 2010.
[9] A. Rebbaa, F. Chu, F. B. Davis, P. J. Davis, and S. A. Mousa, “Novel function of the thyroid hormone analog tetraiodothyrocetic acid: a cancer chemosensitizing and anti-cancer agent,” Angiogenesis, vol. 11, no. 3, pp. 269–276, 2008.
[10] Q.-Z. Long, M. Zhou, X.-G. Liu et al., “Interaction of CCN1 with αvβ3 integrin induces P-glycoprotein and confers vinblastine resistance in renal cell carcinoma cells,” Anti-Cancer Drugs, vol. 24, no. 8, pp. 810–817, 2013.
[11] E. F. Plow, T. A. Haas, L. Zhang, J. Loftus, and J. W. Smith, “Ligand binding to integrins,” Journal of Biological Chemistry, vol. 275, no. 29, pp. 21785–21788, 2000.
[12] J. I. Bergh, H.-Y. Lin, L. Lansing et al., “Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis,” Endocrinology, vol. 146, no. 7, pp. 2864–2871, 2005.
[13] F. B. Davis, H.-Y. Tang, A. Shi h et al., “Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells,” Cancer Research, vol. 66, no. 14, pp. 7270–7275, 2006.
[14] H.-Y. Lin, H.-Y. Tang, A. Shih et al., “Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic,” Steroids, vol. 72, no. 2, pp. 180–187, 2007.
[15] R. Meng, H. Y. Tang, J. Westfall et al., “Crosstalk between integrin αvβ3 and estrogen receptor-α is involved in thyroid hormone-induced proliferation in human lung carcinoma cells,” PLoS ONE, vol. 6, no. 11, Article ID e27547, 2011.
[16] M. K. Luidens, S. A. Mousa, F. B. Davis, H.-Y. Lin, and P. J. Davis, “Thyroid hormone and angiogenesis,” Vascular Pharmacology, vol. 52, no. 3–4, pp. 142–145, 2010.
[17] P. J. Davis, F. B. Davis, S. A. Mousa, M. K. Luidens, and H.-Y. Lin, “Membrane receptor for thyroid hormone: physiologic and pharmacologic implications,” Annual Review of Pharmacology and Toxicology, vol. 51, pp. 99–115, 2011.
[18] S. A. Mousa, H.-Y. Lin, H. Y. Tang, A. Hercbergs, M. K. Luidens, and P. J. Davis, “Modulation of angiogenesis by thyroid hormone and hormone analogues: implications for cancer management,” Angiogenesis, vol. 17, no. 3, pp. 463–469, 2014.
[19] S.-Y. Cheng, J. L. Leonard, and P. J. Davis, “Molecular aspects of thyroid hormone actions,” Endocrine Reviews, vol. 31, no. 2, pp. 139–170, 2010.
[20] Y. Lu, T. Pang, J. Wang et al., “Down-regulation of P-glycoprotein expression by sustained intracellular acidification in K562/Dox cells,” Biochemical and Biophysical Research Communications, vol. 377, no. 2, pp. 441–446, 2008.
[21] J. W. Wojtkowiak, D. Verduzco, K. J. Schramm, and R. J. Gillies, “Drug resistance and cellular adaptation to tumor acidic pH microenvironment,” Molecular Pharmaceutics, vol. 8, no. 6, pp. 2032–2038, 2011.
[22] C.-W. Chou, C.-C. Wang, C.-P. Wu et al., “Tumor cycling hypoxia induces chemoresistance in glioblastoma multiforme by upregulating the expression and function of ABCB1,” Neuro-Oncology, vol. 14, no. 10, pp. 1227–1238, 2012.
[23] Z. Ding, L. Yang, X. Xie et al., “Expression and significance of hypoxia-inducible factor-1 alpha and MDRI/P-glycoprotein in human colon carcinoma tissue and cells,” Journal of Cancer Research and Clinical Oncology, vol. 136, no. 11, pp. 1697–1707, 2010.
[24] I.-S. Hsieh, W.-H. Huang, H.-C. Liou, W.-J. Chuang, R.-S. Yang, and W.-M. Fu, “Upregulation of drug transporter expression by
osteopontin in prostate cancer cells,” *Molecular Pharmacology*, vol. 83, no. 5, pp. 968–977, 2013.

[25] J. M. Yang, G. F. Sullivan, and W. N. Hiat, “Regulation of the function of P-glycoprotein by epidermal growth factor through phospholipase C,” *Biochemical Pharmacology*, vol. 53, no. 11, pp. 1597–1604, 1997.

[26] B. T. Hawkins, D. B. Sykes, and D. S. Miller, “Rapid, reversible modulation of blood-brain barrier P-glycoprotein transport activity by vascular endothelial growth factor,” *The Journal of Neuroscience*, vol. 30, no. 4, pp. 1417–1425, 2010.

[27] C. Riganti, I. Campia, M. Polimeni, G. Pescarmona, D. Ghigo, and A. Bosia, “Digoxin and ouabain induce P-glycoprotein by activating calmodulin kinase II and hypoxia-inducible factor-κ in human colon cancer cells,” *Toxicology and Applied Pharmacology*, vol. 240, no. 3, pp. 385–392, 2009.

[28] R. Liu, Y. Zhang, Y. Chen et al., “A novel calmodulin antagonist O-(4-ethoxy-buty1)-berbamine overcomes multidrug resistance in drug-resistant MCF-7/ADR breast carcinoma cells,” *Journal of Pharmacological Sciences*, vol. 99, no. 7, pp. 3266–3275, 2010.

[29] H.-J. Zhu, J.-S. Wang, Q.-L. Guo, Y. Jiang, and G.-Q. Liu, “Reversal of P-Glycoprotein mediated multidrug resistance in K562 cell line by a novel synthetic calmodulin inhibitor, E6,” *Biological and Pharmacological Bulletin*, vol. 28, no. 10, pp. 1974–1978, 2005.

[30] A. B. Glinkskii, G. V. Glinksky, H.-Y. Lin et al., “Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac),” *Cell Cycle*, vol. 8, no. 21, pp. 3554–3562, 2009.

[31] J. L. Leonard and A. P. Farwell, “Thyroid hormone-regulated actin polymerization in brain,” *Thyroid*, vol. 7, no. 1, pp. 147–151, 1997.

[32] A. P. Farwell, S. A. Dubord-Tomasetti, A. Z. Pietrzykowski, S. J. Stachelek, and J. L. Leonard, “Regulation of cerebellar neuronal migration and neurite outgrowth by thyroxine and 3,3',5'-triiodothyronine,” *Developmental Brain Research*, vol. 154, no. 1, pp. 121–135, 2005.

[33] S. Incperi, P. Luly, P. de Vito, and R. N. Farias, “Short-term effects of thyroid hormones on the Na/H antiport in L-6 myoblasts: high molecular specificity for 3,3',5'-triiodothyronine,” *Endocrinology*, vol. 140, no. 2, pp. 683–689, 1999.

[34] S. D’Arezzo, S. Incperi, F. B. Davis et al., “Rapid nongenomic effects of 3,5,3'-triiodo-L-thyronine on the intracellular pH of L-6 myoblasts are mediated by intracellular calcium mobilization and kinase pathways,” *Endocrinology*, vol. 145, no. 12, pp. 5694–5703, 2004.

[35] J. Lei, C. N. Mariash, M. Bhargava, E. V. Wattenberg, and D. H. Ingbar, “T3 increases Na-K-ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells,” *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 294, no. 4, pp. L749–L754, 2008.

[36] J. Lei and D. H. Ingbar, “Src kinase integrates PI3K/Akt and MAPK/ERK2 pathways in T3-induced Na-K-ATPase activity in adult rat alveolar cells,” *American Journal of Physiology: Lung Cellular and Molecular Physiology*, vol. 301, no. 5, pp. L765–L771, 2011.

[37] S. Incperi, M.-T. Hsieh, H.-Y. Lin et al., “Thyroid hormone inhibition in L6 myoblasts of IGF-I-mediated glucose uptake and proliferation: new roles for integrin αvβ3,” *American Journal of Physiology: Cell Physiology*, vol. 307, no. 2, pp. C150–C161, 2014.

[38] K. M. Mylotte, V. Cody, P. J. Davis, S. D. Blas, and M. Schoenl, “Milrinone and thyroid hormone stimulate myocardial membrane Ca2+-ATPase activity and share structural homologies,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 82, no. 23, pp. 7974–7978, 1985.

[39] P. R. Warnick, F. B. Davis, K. M. Mylotte, P. J. Davis, M. P. Dube, and S. D. Blas, “Calcium channel blocker inhibition of the calmodulin-dependent effects of thyroid hormone and milrinone on rabbit myocardial membrane Ca2+-ATPase activity,” *Biochemical Pharmacology*, vol. 37, no. 13, pp. 2619–2623, 1988.

[40] P. J. Davis, F. B. Davis, and W. D. Lawrence, “Thyroid hormone regulation of membrane Ca2+-ATPase activity,” *Endocrine Research*, vol. 15, no. 4, pp. 651–682, 1989.

[41] A. Breier, L. Gibalova, M. Seres, M. Baranick, and Z. Sulova, “New insight into p-glycoprotein as a drug target,” *Anti-Cancer Agents in Medicinal Chemistry*, vol. 13, no. 1, pp. 159–170, 2013.

[42] T. Mitin, L. L. Von Moltke, M. H. Court, and D. J. Greenblatt, “Levothyroxine up-regulates P-glycoprotein independent of the pregnane X receptor,” *Drug Metabolism and Disposition*, vol. 32, no. 8, pp. 779–782, 2004.

[43] C. Sauvant, M. Nowak, C. Wirth et al., “Acidosis induces multidrug resistance in rat prostate cancer cells (AT1) in vitro and in vivo by increasing the activity of the p-glycoprotein via activation of p38,” *International Journal of Cancer*, vol. 123, no. 11, pp. 2532–2542, 2008.

[44] O. Thews, B. Gassner, D. K. Kelleher, G. Schwerdt, and M. Gekle, “Impact of extracellular acidity on the activity of P-glycoprotein and the cytotoxicity of chemotherapeutic drugs,” *Neoplasia*, vol. 8, no. 2, pp. 143–152, 2006.

[45] M. Baudouin-Legros, F. Brouillard, D. Tondelier, A. Hinzpeter, and A. Edelman, “Effect of ouabain on CFTR gene expression in human Calu-3 cells,” *The American Journal of Physiology*, vol. 284, no. 3, pp. C620–C626, 2003.

[46] T. Ishimaru, Y. Watanabe, S. Kita, and M. P. Blaustein, “Na+/Ca2+ exchange inhibitors: a new class of calcium regulators,” *Cardiovascular & Hematological Disorders-Drug Targets*, vol. 7, pp. 188–198, 2007.

[47] A. Shih, S. Zhang, H. J. Cao et al., “Disparate effects of thyroid hormone on actions of epidermal growth factor and transforming growth factor-α are mediated by 3,5'-cyclic adenosine 5'-monophosphate-dependent protein kinase II,” *Endocrinology*, vol. 145, no. 4, pp. 1708–1714, 2007.

[48] H. Y. Lin, A. Shih, F. B. Davis, and P. J. Davis, “Thyroid hormone promotes the phosphorylation of STAT3 and potentiates the action of epidermal growth factor in cultured cells,” *Biochemical Journal*, vol. 338, no. 2, pp. 427–432, 1999.

[49] A. Seidel, A. Bunge, B. Schaefer et al., “Intracellular localization, vesicular accumulation and kinetics of daunorubicin in sensitive and multidrug-resistant gastric carcinoma EPG85-257 cells,” *Virchows Archiv*, vol. 426, no. 3, pp. 249–256, 1995.

[50] H. Y. Lin, M. Sun, H. Y. Tang et al., “L-thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase,” *The American Journal of Physiology—Cell Physiology*, vol. 296, no. 5, pp. C980–C991, 2009.

[51] S. Reza, A. Shaukat, T. M. Arain, Q. S. Riaz, and M. Mahmud, “Expression of osteopontin in patients with thyroid dysfunction,” *PLoS ONE*, vol. 8, no. 2, Article ID e56533, 2013.

[52] L. Liu, X. Ning, L. Sun et al., “Hypoxia-inducible factor-κ contributes to hypoxia-induced chemo-resistance in gastric cancer,” *Cancer Science*, vol. 99, no. 1, pp. 121–128, 2008.
[53] L. Min, Q. Chen, S. He, S. Liu, and Y. Ma, "Hypoxia-induced increases in A549/CDDP cell drug resistance are reversed by RNA interference of HIF-1α expression," *Molecular Medicine Reports*, vol. 5, no. 1, pp. 228–232, 2012.

[54] M. Saeki, K. Kurose, R. Hasegawa, and M. Tohkin, "Functional analysis of genetic variations in the 5'-flanking region of the human MDR1 gene," *Molecular Genetics and Metabolism*, vol. 102, no. 1, pp. 91–98, 2011.

[55] L.-Y. Chiu, J.-L. Ko, Y.-J. Lee, T.-Y. Yang, Y.-T. Tee, and G.-T. Sheu, "L-type calcium channel blockers reverse docetaxel and vincristine-induced multidrug resistance independent of ABCB1 expression in human lung cancer cell lines," *Toxicology Letters*, vol. 192, no. 3, pp. 408–418, 2010.

[56] I. Villanueva, C. Alva-Sánchez, and J. Pacheco-Rosado, "The role of thyroid hormones as inducers of oxidative stress and neurodegeneration," *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 218145, 15 pages, 2013.

[57] D. Gnocchi, S. Leoni, S. Incerp, and G. Bruscalupi, "3,5,3'-Triiodothyronine (T₃) stimulates cell proliferation through the activation of the PI3K/Akt pathway and reactive oxygen species (ROS) production in chick embryo hepatocytes," *Steroids*, vol. 77, no. 6, pp. 589–595, 2012.

[58] Y. Cai, J. Lu, Z. Miao, L. Lin, and J. Ding, "Reactive oxygen species contribute to cell killing and P-glycoprotein downregulation by salvicine in multidrug resistant K562/A02 cells," *Cancer Biology & Therapy*, vol. 6, no. 11, pp. 1794–1799, 2007.

[59] M. D. Hall, M. D. Handley, and M. M. Gottesman, "Is resistance useless? Multidrug resistance and collateral sensitivity," *Trends in Pharmacological Sciences*, vol. 30, no. 10, pp. 546–556, 2009.

[60] H.-Y. Lin, H. Y. Tang, F. B. Davis et al., "Nongenomic regulation by thyroid hormone of plasma membrane ion and small molecule pumps," *Discovery Medicine*, vol. 14, no. 76, pp. 199–206, 2012.

[61] F. J. Sharom, "The P-glycoprotein multidrug transporter," *Essays in Biochemistry*, vol. 50, no. 1, pp. 161–178, 2011.

[62] A. M. Mitchell, M. Tom, and R. H. Mortimer, "Thyroid hormone export from cells: contribution of P-glycoprotein," *Journal of Endocrinology*, vol. 185, no. 1, pp. 93–98, 2005.

[63] R. R. Cavalieri, L. A. Simone, S. W. Park et al., "Thyroid hormone export in rat FRTL-5 thyroid cells and mouse NIH-3T3 cells is carrier-mediated, verapamil-sensitive, and stereospecific," *Endocrinology*, vol. 140, no. 11, pp. 4948–4954, 1999.

[64] D. Fu, "Where is it and how does it get there—intracellular localization and traffic of P-glycoprotein," *Frontiers in Oncology*, vol. 23, article 321, 2013.

[65] H. J. Cao, H.-Y. Lin, M. K. Luidens, F. B. Davis, and P. J. Davis, "Cytoplasm-to-nucleus shuttling of thyroid hormone receptor-β1 (TRβ1) is directed from a plasma membrane integrin receptor by thyroid hormone," *Endocrine Research*, vol. 34, no. 1-2, pp. 31–42, 2009.

[66] H. Burger, W. J. Loos, K. Eechoute, J. Verweij, R. H. J. Mathijssen, and E. A. C. Wiemer, "Drug transporters of platinum-based anticancer agents and their clinical significance," *Drug Resistance Updates*, vol. 14, no. 1, pp. 22–34, 2011.