Steroid/Thyroid Receptor-like Proteins with Oncogenic Potential: A Review

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Abstract

Mutated or truncated forms of certain members of the steroid/thyroid receptor superfamily have oncogenic potential. The aberrant forms compete with the normal receptor for binding to the responsive element on the DNA and thus interfere negatively with the normal transcription control mechanism. Oncogenes that arise from dominant negative mutations may therefore be called "donones," to distinguish them from recessive types such as that causing retinoblastoma ("renoncs"). It is possible that donones are also responsible for the loss of hormonal responsiveness of some tumors during progression.

Research during the past few years has revealed the existence of a class of genes that encode ligand-dependent DNA-binding proteins with a zinc-binding "finger" motif. Because the steroid and thyroid receptors belong to this class, it has been called the "steroid/thyroid receptor superfamily." It is not the purpose of this article to give a complete review of this gene superfamily, inasmuch as excellent reviews have already been published by other authors (cf. Refs. 1–3), but rather to look at certain members that may be involved in malignant transformation and/or tumor progression.

General Structure of Steroid/Thyroid Receptors

In 1985 Sluyser and Mester (4) proposed that the products of certain oncogenes may bear structural resemblance to steroid hormone receptors. This idea was based on the hypothesis that the loss of hormonal dependency of certain tumors might be due to the appearance of mutated or truncated steroid receptor-like proteins that act constitutively, i.e., enhance transcriptional activity even in the absence of hormone. These aberrant proteins would be either produced due to mutations in steroid receptor genes or encoded by oncogenes that have homology with steroid receptor genes (4).

Subsequently, investigations in several laboratories show that steroid receptors have structural homology with the avian erythroblastosis virus oncogene v-erb-A.

Steroid hormone receptor proteins contain different domains which have been designated A–F (1, 2). The assignment of these domains is based on degrees of homology between steroid receptors; i.e., regions A, C, and E are highly conserved between these receptors, whereas this is less the case for regions B, D, and F. Subsequent studies revealed that these regions serve different functions. Domain C contains two zinc-binding "fingers" that interact with DNA. Domain E is the region to which the hormone molecule binds. The D region separating C and E has been called "hinge region" because it is thought to act as a hinge between the DNA-binding and hormone-binding domains. The A/B domain may serve to modulate the receptor-DNA interactions and also may cause hormone-independent trans-activation (see below). Thyroid hormone receptors also have this general structure, and the superfamily also includes genes for retinoic acid receptors, vitamin D3 receptors, and dioxin receptors. More members of this superfamily are likely to exist (3). The genomic organization of these genes probably follows the pattern found for the progesterone and estrogen receptor genes. These genes are split into 8 exons. The A/B region is almost entirely encoded within a single exon, and each of the putative "zinc fingers" of region C is encoded separately. Region E is assembled from 5 exons (5, 6). Some members of the steroid/thyroid hormone receptor superfamily are shown in Fig. 1.

Viral erb-A and Thyroid Hormone Receptors

Viral erb-A was originally discovered when it was found that the ability of avian erythroblastosis virus to transform erythroblasts is determined by two oncogenes, v-erb-A and v-erb-B (7–9). Both erb-A and erb-B oncogenes are homologous to avian and mammalian chromosomal DNA sequences c-erb-A and c-erb-B (10). Cellular erb-B is a truncated form of the epidermal growth factor receptor (11). The erb-A oncogene potentiates the transforming activity of erb-B and appears to be responsible for the early blockage of cell differentiation within the erythroid lineage (12). In transfection experiments, the v-erb-A oncogene product by itself is sufficient to transform erythrocyte cells (13). The human (14) and chicken (15) c-erb-A genes, the cellular counterparts of the viral oncogene v-erb-A, represent thyroid hormone (T3) receptors.

The erb-A genes encode a cysteine-rich domain that shows high homology with the putative DNA-binding domain of steroid hormone receptors (13–20). The amino acid sequence of this domain in v-erb-A is almost identical to that of the glucocorticoid receptors (21, 22) and estrogen receptors of various species (23, 24). A thyroid hormone receptor called erb-A-T has been described for the human testis which is closely related to the chicken erb-A (25).

Interestingly, several c-erb-A-related genes exist in the human genome (26). Of the multiple human homologues to v-erb-A the most closely related homologue is the hc-erb-A1 gene on human chromosome 17 (27) that probably encodes erb-A-T. Another homologue, the hc-erb-AB gene, is located on human chromosome 3 (13).

The existence of multiple thyroid hormone receptors suggests that these receptors play different roles from one tissue to the other. This is confirmed by studies in the rat where of 3 thyroid hormone receptors described, one (called r-erb-Aβ-2) is expressed only in the pituitary gland (28) whereas other homologues of the v-erb-A oncogene are expressed in other tissues. Of interest is that alternative splicing generates messages encoding rat c-erb-A proteins that do not bind thyroid hormone (29) (Table 1).

The thyroid hormone receptor, in the absence of its ligand, blocks the activity of a responsive promoter. This suppression is abolished when thyroid hormone is added to the system, which stimulates expression. The oncogenic analogue of the
thyroid hormone receptor, v-erb-A, acts as a constitutive repressor in this system and, when coexpressed with the receptor, blocks activation (30).

The v-erb-A product has a lower affinity than the normal receptor for binding to a thyroid hormone-responsive element in the long terminal repeat of Moloney murine leukemia virus, which binds c-erb-A protein. Overexpressed v-erb-A protein may therefore interfere negatively with normal transcriptional mechanisms (31), suggesting that in fact v-erb-A is a transcriptional regulator rather than a normal thyroid hormone receptor superfamily. Primary amino acid sequences have been aligned on basis of regions of maximum amino acid similarity. Amino acid numbers are those for the human receptors with the exception of v-erb-A. GR, glucocorticoid receptor; MR, mineralocorticoid receptor; VDR, vitamin D3 receptor; T3R, thyroid hormone receptor; RAR, retinoic acid receptor; HAP, hepatoma-associated protein (epithelial type retinoic acid receptor, RAR£). The DNA-binding regions (region C) are shaded (1-3).

Table 1 Cellular homologue to the v-erb-A oncogene in the rat

<table>
<thead>
<tr>
<th>Homologue</th>
<th>T3 binding</th>
<th>Expression</th>
</tr>
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<tbody>
<tr>
<td>r-erb-Aa-1</td>
<td>+</td>
<td>Skeletal muscle, brown fat</td>
</tr>
<tr>
<td>r-erb-Aa-z</td>
<td>-</td>
<td>Brain, hypothalamus</td>
</tr>
<tr>
<td>r-erb-Aa-2z</td>
<td>+</td>
<td>Kidney, liver</td>
</tr>
<tr>
<td>r-erb-Aa-2</td>
<td>+</td>
<td>Pituitary gland</td>
</tr>
</tbody>
</table>

* r-erb-Aa-2 is a truncated form of the thyroid hormone receptor that lacks the thyroid hormone (T3) binding domain. Data taken from Ref. 29.

Glucocorticoid Receptor Mutants

The glucocorticoid receptor has been cloned (20), and the functions of various domains have been analyzed (37-39). The amino acid residues involved in attachment of the ligand have been identified (40). Two separate sequences within the glucocorticoid receptor, NL1 and NL2, act as signals for the hormone-dependent nuclear localization of the receptor (41). The carboxyl terminus of the human receptor contains a 30-amino acid sequence (named "r2") that functions as an activation domain. A similar and independent activity has also been identified in the amino-terminal region of the receptor. These two sequences in the molecule are both acidic but are structurally unrelated (42).

Removal of 29 amino acids from the carboxy terminus of the rat glucocorticoid receptor leads to only very little (1%) loss of hormone-inducible activity, but further deletions cause loss of hormone-inducible activity (39, 43). While deletions up to 180 carboxy-terminal residues fail to produce a biological response in either the presence or the absence of steroid, longer carboxy-terminal deletions or truncation of the amino-terminal part of the domain induce transcriptional activation even in the absence of hormone. Truncation at both ends of the molecule resulted in a polypeptide of only 150 amino acids which is still effective in constitutive activation (43).
Regions in the carboxy and amino termini of the glucocorticoid receptor that increase transcription but are not involved in DNA binding may be moved to other parts of the receptor or attached to heterologous binding domains and still maintain function by increasing transcription (42). Such studies can also be done using systems in which glucocorticoid receptors exert negative effects. The results suggest that the negative effects on transcription that glucocorticoid receptors exert in some systems are generated via steric hindrance. The amino terminus is not critical for this repression but both the DNA- and hormone-binding domains are required for efficient repression to occur (44). These data on receptor structure and function are of interest because certain cells (e.g., mouse T-cell lymphomas and human lymphoblastic leukemia cells) are known to contain mutant glucocorticoid receptors with structural defects which causes an inability to mediate in glucocorticoid-induced cell lysis. Because growth of the normal ("wild-type") cells is inhibited by adding glucocorticoids, this makes isolation of glucocorticoid-resistant cell variants quite easy. The majority of these resistant cells possess glucocorticoid receptors with structural and functional defects. Four major abnormalities have been identified: r~ (receptor deficient), nt~ (nuclear transfer deficient), nt1 (nuclear transfer increased), and act1 (activation labile). The r~ phenotype occurs most often and is characterized by low or undetectable hormone binding. Some r- cells contain a polypeptide that cross-reacts with anti-receptor antibodies and has the same molecular weight as the wild-type receptor (M, 94,000). This suggests a mutation in the hormone-binding domain (45–47). The r~ phenotype may in some cells also arise from lack of expression of receptor with no gene product or specific mRNA detectable (48). Whatever the cause of the r- phenotype may be, this phenomenon is not due to gross DNA rearrangements or deletions and therefore perhaps may be due to point mutations (49).

The glucocorticoid receptor of the nt phenotype is a M, 40,000 polypeptide that represents the amino-terminally truncated form of the wild-type receptor. These nt receptors are synthesized as shorter polypeptides rather than being processed from larger molecules. The mRNA of these truncated receptors is about 1.5 kilobases shorter than the wild-type mRNA from which the 5’ sequences are missing. However, Southern blot analysis did not reveal any genomic deletions or rearrangements (47, 49). Therefore it seems likely that transcription is initiated correctly and that aberrant splicing is responsible for the truncated nt mRNA (38).

Receptors of the act1 phenotype are relatively labile in the sense that the hormone is dissociated easily when these receptors are activated. The act1 receptors, however, behave in a manner similar to that of wild-type receptors if the hormone is attached covalently by cross-linking labeling and thus unable to dissociate upon activation of the complex (50).

Glucocorticoid receptors have also been studied at the protein level. Mild treatment with proteases generates fragments that structurally resemble the nt receptors of glucocorticoid-resistant cells and that resemble these nt receptors in exhibiting increased nonspecific affinity for DNA in vitro. However, this does not mean that these molecules can activate transcription since other studies show that an intact amino terminus (A/B region) is required for the glucocorticoid receptor to be able to activate efficiently the HRE of the MMTV-LTR (37, 51).

Analysis of the normal rat liver glucocorticoid receptor protein indicates that the amino terminus is blocked (52). Glucocorticoid receptors are phosphoproteins (53–55), and serine (53) or threonine (55) residues are phosphorylated depending on the conditions. Whether these phosphorylations serve a role in receptor functioning, and if so in what way, is unclear at present (56).

Steroid receptors that are isolated from cytosols are often found to be complexed with M, 90,000 heat shock protein (57). There is a debate whether these complexes serve a role in steroid hormone action or are just artifacts of the isolation procedure. Whatever the answer may be, phosphorylation of the receptor does not appear to play a role in the association or dissociation of glucocorticoid receptor from M, 90,000 heat shock protein (58). When rat thymus cells are depleted of ATP by anaerobiosis, the specific glucocorticoid-binding capacity of these cells disappears, and it rapidly reappears when ATP levels are restored. In cells deprived of ATP the glucocorticoid receptor is present in a "null receptor" form that cannot bind hormone and that is bound in the nuclei of the ATP-depleted cells. It is possible that the null receptor is present as the dominant form in r- mutant cells such as described above (59).

**Progesterone Receptor A and B Forms**

PRs in chicken and human tissues are represented by two molecular forms, A and B, with molecular weights of 79,000 and 109,000, respectively. Equimolar ratios of these A and B forms have been found in the cytosols of chicken oviduct tubular gland cells and in human breast cancer T-47D cells (60, 61). Immunoanalysis and peptide mapping of the photoaffinity-labeled proteins indicate that the A and B forms are structurally related (60, 62, 63). Furthermore, both forms bind to DNA (62). This finding has been confirmed by DNasel footprinting experiments using the hormone-responsive element of the chicken lysozyme gene (64). Expression of the cloned cDNA produced a protein that resembles the natural B form (M, 109,000).

A protein corresponding in size to form A (M, 79,000) was produced by expressing an amino-terminally truncated receptor starting at Met-128 or by internal initiation during in vitro translation (65, 66).

Evidence has been presented that the A and B proteins are derived by alternate initiation of translation from a single mRNA transcript (67). Comparison of the sequences surrounding the AUG triplets in rabbit (68), human (65), and chicken (69) PRs indicates only partial conservation and does not provide an explanation for only a single form of PR (the B form) in rabbit, in contrast to the two protein forms (A and B) found in humans and chickens. It has therefore been proposed that the secondary structural context of these sequences on the mRNA may play a role in determining whether alternate initiation of translation will take place (67).

The A/B region of the chicken progesterone receptor plays a crucial role in the differential activation by progesterone of the ovalbumin gene and the MMTV-HRE. This is shown by the fact that whereas progesterone receptor form B preferentially activates the MMTV-LTR promoter, its naturally occurring amino-terminally truncated form (form A) can activate the promoter of both genes (70).

The chicken progesterone receptor gene appears to be present in a single copy and to consist of a 38-kilobase transcription unit (6). An alternative polyadenylation signal is present near the 5’ end of the second intron; this results in a truncated mRNA. The putative protein product of this variant (1.8 kilobases) mRNA would contain only the amino-terminal region and one-half of the DNA-binding region. If actually present in cells, such truncated receptors might compete with normal
receptors for available steroid-regulatory elements on target genes. Alternatively, such molecules may exist as dangerous cellular variants if they retain any biological activity, since the repressive hormone-binding regulatory domain is absent (6).

Estrogen Receptor Variants

The hER gene has been mapped to human chromosome 6 (3, 71), and the murine ER gene to mouse chromosome 10 (72). Comparison of the ERs of various species reveals complete (100%) homology between the DNA-binding domains of these species (16, 24, 73, 74). The ER of the cold-blooded organism Xenopus laevis exhibits high similarity in amino acid sequence with the human and avian ERs. In the putative DNA-binding region, its amino acid sequence differs at only 1 of 83 amino acids (75).

The DNA-binding region (region C) plays a role in specific recognition and binding to the estrogen-responsive elements of target genes, whereas region E is indispensable for hormone binding. Receptors that lack the hormone-binding domain, however, still recognize specific responsive DNA elements, while the isolated hormone-binding domain binds estradiol with wild-type affinity (23, 76). The length of the hinge region D, while the isolated hormone-binding domain binds estradiol with activation function is generated by the three-dimensional fold of the hER in GAL-4 binding sites in the absence of hormone (or anti-hormone)-dependent binding to nuclei, and are not heat-shock dependent receptors (24, 73). The wild-type affinity (23, 76). The length of the hinge region D, while the isolated hormone-binding domain binds estradiol with activation function is generated by the three-dimensional fold of the hER in GAL-4 binding sites in the absence of hormone (or anti-hormone)-dependent binding to nuclei, and are not heat-shock dependent receptors (24, 73).

In order to elucidate the role of the hormone-binding domains of hER and human glucocorticoid receptor, these carboxy-terminally located sequences were joined to the DNA-binding domain of the yeast transcription factor GAL-4 (77). Stimulation of transcription by these chimeric receptors from GAL-4-responsive reporter genes was hormone dependent. The chimeric receptor only bound tightly to nuclei when hormone or antihormone was present. However, the antihormone did not cause transcription activation, indicating that the hormone-binding region of receptors has a dual function by both causing hormone (or anti-hormone)-dependent binding to nuclei, and hormone (but not antihormone)-dependent activation of transcription.

Of interest also was that GAL-ER [147-251] binds tightly to nuclei even in the absence of hormone and that it competes for GAL-4-activated transcription. Apparently, the ER sequence 147-251 does not efficiently mask the DNA-binding domain in GAL-ER [147-251]; hence this chimeric receptor can by its own accord attach to the GAL-4 binding sites in the absence of hormone (77).

The transcriptional activation function located in the hormone-binding domain of hER is encoded by separate codons. The results suggest that the protein surface responsible for the activation function is generated by the three-dimensional folding of the hormone-binding domain and is most likely created from dispersed elements (78). This finding is of interest because the location of transcriptional activation domains within steroid receptors have been controversial. Whereas receptors that are deleted for the hormone-binding domain do not affect constitutive activation, the magnitude of this activation ranges from only 5% to full wild-type activity depending on the receptor and the experimental conditions. This indicates that the NH2-terminal A/B region is important for activation, an idea that is also supported by the fact that NH2-terminal deletions of the hER (70, 76) and chicken PR (69, 79) can reduce the ability of the receptor to activate transcription, but only on certain promoters, suggesting that the A/B region may be interacting with promoter-specific factors.

It has been reported that the human ER cDNA clone pOR8 obtained from MCF-7 cells contains a glycine 400 (GGG) to valine 400 (GTG) mutation compared to the ER of a human genomic library (5). In addition, two silent mutations (Ser-10, TCC - TCT; Thr-594, ACG - ACA) are present in the MCF-7 ER cDNA. Interestingly, a glycine is found at position 400 in the chicken ER (74), mouse ER (24), rat ER (73), and Xenopus ER (75) sequences suggesting that it is the MCF-7 ER which differs from the wild-type sequence. The Gly-400 Val-400 mutation is in the hormone-binding domain of the cloned hER and destabilizes the ER structure, thereby decreasing its apparent affinity for estradiol at 25°C, but not at 4°C. This point mutation may be a cloning artifact, because the valine codon GTG was not found in the sequence of three other hER cDNA clones derived from MCF-7 cell RNA. However, it is also possible that a minor population of MCF-7 cells had this mutation or that a minor fraction of hER mRNA is mutated posttranscriptionally (80). RFLP has been reported in ER genes. With restriction enzyme PstI, absence of a 0.6-kilobase restriction fragment was found to be associated with ER-negative human breast cancer cells; instead the ER-negative cells had a restriction fragment of 1.6 kilobases. The RFLP is probably located within sequences encoding the DNA- or hormone-binding regions (81). The finding that ER-positive and ER-negative breast tumors differ significantly in RFLP is of interest since it suggests that ER activation or lack of activation is associated with different alleles (designated P1 and P2). The molecular basis of this phenomenon is unknown, but it might affect the proper splicing of ER mRNA. There is the possibility of point mutations being responsible for the effect. Perhaps sequence analysis of ER mRNA from breast cancer patients may elucidate this matter (81).

PstI restriction enzyme analysis has identified a single two-allele polymorphism in which the 1.4-kilobase allele occurs in 91% of North American Caucasians and the 1.7-kilobase allele in 9% (82).

Multiple ER mRNA variants have been reported for X. laevis. The Xenopus ER encodes 4 mRNAs approximately 9, 6.5, 2.8, and 2.5 kilobases long. It is possible that these derive from different polyadenylation signals (75). Transcription initiation sites at 10 major sites have been reported close together for the mouse ER gene; however, in the mouse uterus only a single ER mRNA transcript is observed (24). Of interest is that when the mouse ER cDNA is tested for functional activity by transfection, it shows not only hormonally dependent activity but also some constitutive activity. The latter may possibly be due to the presence of mutated or truncated receptors (24, 83).

The transcripts required to encode a protein the size of the human ER protein (M, 65,000) is about 2 kilobases. In human uterus the size of the ER mRNA is, however, found to be about 4.2 kilobases, and therefore about one-half of the mRNA appears to be untranslatable (82). This is also the case with the ER mRNA of MCF-7 human breast cancer cells which show a short 5' and a very long 3' untranslatable region (16, 17).

The degree of transcriptional stimulation by truncated mutants of human ER is dependent on cell type and promoter context (84).

ER Proteins

Studies at the protein level have revealed ER variants in various tissues. Such studies generally involve covalent labeling of cytosolic ER with [3H]tamoxifen aziridine in the presence of protease inhibitors and subsequent identification of the labeled
receptors by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Investigations of this type carried out with mouse uterus demonstrated a major ER component (M, 65,000) with minor fragments with molecular weights of 54,000 and 37,000. Perhaps the low molecular weight forms originate from the M, 65,000 holoreceptor by proteolytic degradation, but the possibility that the M, 65,000, 54,000, and 37,000 species are different gene products cannot be ruled out (85). Similar experiments in our laboratory using [3H]tamoxifen aziridine tagging of estrogen-dependent and -independent GR mouse mammary tumors show essentially similar results. In these tumors we detected M, 65,000, 50,000, and 35,000 receptors. Of interest was that there was a shift towards the low molecular weight forms when these mouse tumors became hormonally independent during serial transplantations in syngeneic mice.2

A protease with α-chymotrypsin activity that increased the affinity of ER for binding to DNA has been described in mammary tumors of C3H mice (86). However, there is also evidence for a specific hormonally regulated modification of ER. The response, as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is the formation of a closely spaced ER doublet (M, 65,000 and 66,500) in nuclei of the mouse uterus (87). A possible explanation for the doublet might be a phosphorylative mechanism, inasmuch as it has been reported that ER from calf uteri is phosphorylated and dephosphorylated by a nuclear kinase and a cytosolic phosphatase (88). It should be pointed out that even if some of the low molecular weight forms of ER observed in some tissues turn out to be proteolytic cleavage products of the holoreceptor, this proteolysis may have functional significance. The cleavage is at the border regions of the functional domains and may serve to separate these regions. Furthermore, it has been reported that the ER molecule itself has proteolytic activity that is responsible for its own transformation to the active state (89).

Loss of Hormonal Dependence

The loss of hormonal dependence in breast cancer is due to the emergence of autonomous cell clones that progressively achieve dominance in the tumor mass (90, 91). This apparent heterogeneity of the tumors suggests that combined chemohormonal therapy would be a more effective treatment for breast cancer than either single modality alone. Animal studies indicate this to be the case; however, these studies also show that even if the combined treatment does not cure but only extends the latency period of the tumor (92, 93). This is also observed in human studies where the combined approach causes higher response rates and longer relapse-free intervals than either modality used singly. Even with chemoendocrine combinations, however, complete remissions remain relatively uncommon and cure of metastatic diseases remains impossible (94). The reason for this disappointing result apparently is that the cytotoxic drugs used in studies thus far are not sufficiently effective in destroying the highly malignant subclones that emerge during the tumor progression. It seems likely that breast cancer progression is a highly complex affair, resulting in the emergence of increasingly malignant cells by natural selection. The question therefore is, "Where do these cells come from and what is the mechanism by which they are able to achieve dominance?"

Assuming that a breast tumor originates from a single transformed breast tissue cell, it seems likely that the subsequent outgrowth of the tumor and the progression steps involves different oncogenes. Several oncogenes have been implicated in human mammary cancer including myc, ras, src, neu, and perhaps others (95). Many of the chromosomes frequently altered in human breast cancer (i.e., chromosomes 1, 3, 6, 7, and 11) contain sequences which encode human cellular oncogenes. Examples of this are c-bi1m, N-ras, and c-ski on chromosome 1; c-rat on chromosome 3; c-myb on chromosome 6; c-erbB and c-met on chromosome 7; and H-ras and c-ets on chromosome 11 (96). It has been reported that c-myb expression shows a strong association with human breast cancers that have high estrogen receptor levels (97). The myb gene, like the ER gene, is on human chromosome 6 (98) and mouse chromosome 10 (72). Whether these genes are within the same region on these chromosomes is not known. If this turns out to be the case, it raises the interesting possibility that the myb oncogene is involved in the mechanism by which levels of ER are enhanced in hormone-dependent breast cancers. Kasid et al. (99) have reported that when human breast cancer MCF-7 cells were transfected with v-Ha-ras, these cells no longer responded to exogenous estrogens in culture and were fully tumorigenic without estrogen supplementation when tested in nude mice. These transformed cells still had high levels of ER. The authors concluded that MCF-7 cells that acquire an activated ras gene can bypass the hormonal regulatory signals that trigger the neoplastic growth of the breast cancer cell line. Interestingly, transfection of the v-Ha-ras oncogene into MCF-7 cells causes these cells to secrete increased levels of several growth factor activities constitutively, suggesting that growth of hormonally independent breast cancer cells might be stimulated by these growth factors (100). Cultured ER-negative cell lines constitutively secrete relatively high concentrations of several growth factors such as transforming growth factor α, insulin-like growth factor I, platelet-derived growth factor, an epithelial cell colony-stimulating factor, mammary-derived growth factor, and autocrine motility factor (95). Secretion of several of these growth factor activities in ER-positive lines is regulated by estrogen: estrogen deprivation or antiestrogen treatment reduces the growth factor secretion; whereas estrogen administration increases it (95). Thus, ER-negative cells might have a growth advantage due to constitutive growth factor secretion. However, results by Osborne et al. (101) are at variance with this hypothesis. These authors inoculated ER-negative MDA-231 human breast cancer cells in castrated female athymic nude mice and found that the resulting tumors did not support growth of MCF-7 cells inoculated in the opposite flank, which required estrogen supplementation for growth. In this model system, growth factors therefore were not capable of replacing estrogen for growth stimulation of MCF-7 breast cancer cells. These data make it seem unlikely that the loss of estrogen dependence in breast cancer can be explained simply by the constitutive production of growth factors that replace estrogen in stimulation of cell growth. If therefore growth factors are not responsible for sustaining hormone-independent growth, how is this growth sustained? We have proposed that mutated and/or truncated steroid receptor-like proteins act as constitutive activators (4). Of interest is the fact that estrogen receptor variants have been reported in human mammary cancers and that the relative amounts of these proteins increase with loss of hormonal dependency (102–104).

Conclusions

In recent years we have seen a tremendous revolution in the field of steroid hormone action. The exciting discovery of the
relationship between the structures of steroid and thyroid receptors with that of the viral oncogene v-erb-A makes it tempting to speculate that activation of some of these receptor genes is implicated in oncogenesis.

That v-erb-A can exert its specific effects on erythroblasts even though it cannot bind thyroid hormones apparently is due to changes in hormone binding domain E of c-erb-A having occurred. It seems possible that these mutations prevent the E region from masking the DNA-binding region (domain C), thereby permitting constitutive binding of the receptor to DNA.

In the progesterone receptor gene there is an alternative polyadenylation signal near the 5' end of the second intron, and this could result in a truncated mRNA. The putative protein product of this variant (1.8 kilobases) mRNA would contain only the NH2-terminal domain and one-half of the DNA-binding domain of the complete receptor and would lack hormone-binding activity. If actually present in cells, such truncated receptors might compete with normal receptor forms for available steroid-regulatory elements on target genes and thus have oncogenic potential (86). Of interest also is the difference between the complete progesterone receptor (form B) and its truncated form (form A) in its ability to activate specific genes (70).

The question can be raised whether the low molecular weight forms of steroid receptors that have been found in various tissues serve a physiological function. It is important to establish which of these forms are due to changes at the genomic or mRNA level and which are caused by secondary events, such as phosphorylation or proteolysis. Proteases have been reported that can degrade estrogen and progesterone receptors selectively (105). Steroid receptors are particularly susceptible to proteases in tissues serving a physiological function. It is important to establish which of these forms are due to changes at the genomic or mRNA level and which are caused by secondary events, such as phosphorylation or proteolysis.

The presence of abnormal ER variants in breast cancer (102, 103, 107) suggests that a subclassification of tumors based on functional abnormalities of ER may predict refractoriness to hormone therapy. Abnormal methylation of the ER gene reduces ER mRNA levels in human endometrial carcinomas (108) and this may be the case in breast cancers.

It has been reported that malignant human prostatic tissue contains a 4–5S androgen receptor form that is not present in normal prostate (104). Besides being involved in liver carcinogenesis (hap gene) and induction of avian leukemia (v-erb-A), certain members of the steroid/thyroid receptor family may play a role in other specific types of cancer. Changes in the coding region of the DNA-binding domain of glucocorticoid receptors have been reported between mouse lymphoma cells and an androgen-dependent mouse mammary tumor (109). Therefore, changes in steroid receptors at the genomic level may be associated with certain malignant states.

Deletions of chromosome 3 have been reported in SCLC and many non-small cell tumors of the lung (110). In the case of SCLC a consensus deletion (3p21→25) has been defined which contains a putative suppressor gene (111). The thyroid hormone receptor \( \text{T}_{3}\text{R}_{\beta} \) (c-erb-A\( \beta \)) locus maps to this region on chromosome 3, and the \( \text{erb-A}\beta \) hybridization to DNA extracted from these tumors is reduced, suggesting that either \( \text{T}_{3}\text{R}_{\beta} \) or another erb-A-related gene found at this locus may be the suppressor gene. It is also possible that another suppressor gene is located at this locus, perhaps the \( \text{RAR}_{\beta} \) gene which maps to chromosome 3p24 (112). It has been proposed that in SCLC, both alleles of the c-erb-A gene are inactivated, one by a chromosomal deletion and the other by a more subtle mutation.

Donons

Herskowitz (113) has proposed that some oncogenes may be examples of naturally occurring dominant negative mutations. Both dominant and recessive mutations are known to cause cellular transformation. Recessive mutations, such as that causing retinoblastoma, involve loss of wild-type functions. By contrast, v-erb-A may be an example of a dominant negative mutation, inasmuch as this oncogene acts as a constitutive repressor and, when coexpressed with the thyroid-hormone receptor, blocks activation of the thyroid hormone-regulated gene (30). Thyroid hormone action is also inhibited by a non-hormone-binding form of the c-erb-A protein that is generated by alternative mRNA splicing (29, 114).

Oncogenes that arise from dominant negative mutations may therefore be called "donons," to distinguish them from recessive types such as that causing retinoblastoma ("renoncs"). In conclusion, steroid receptors have moved from the role of prognostic guides and guides to therapy into a new role, that of potential pathogenic factors in oncogenesis. In the next few years we can expect to be able to define the structures and function of the abnormal transcripts of these and other DNA-binding "finger" proteins and to establish their roles in oncogenesis and tumor progression in specific types of cancer.

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Steroid/Thyroid Receptor-like Proteins with Oncogenic Potential: A Review

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