Dopamine Receptors in Breast Cancer: Prevalence, Signaling, and Therapeutic Applications

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ABSTRACT: Breast cancer (BC) is the most common malignancy among women, with over one million cases occurring annually worldwide. Although therapies against estrogen receptors and HER2 have improved response rate and survival, patients with advanced disease, who are resistant to anti-hormonal therapy and/or to chemotherapy, have limited treatment options for reducing morbidity and mortality. These limitations provide major incentives for developing new, effective, and personalized therapeutic interventions. This review presents evidence on the involvement of dopamine (DA) and its type 1 receptors (D1R) in BC. DA is produced in multiple peripheral organs and is present in the systemic circulation in significant amounts. D1R is overexpressed in ~ 30% of BC cases and is associated with advanced disease and shortened patient survival. Activation of D1R, which signals via the cGMP/PKG pathway, results in apoptosis, inhibition of cell invasion, and increased chemosensitivity in multiple BC cell lines. Fenoldopam, a peripheral D1R agonist that does not penetrate the brain, dramatically suppressed tumor growth in mouse models with D1R-expressing BC xenografts. It is proposed that D1R should serve as a novel diagnostic/prognostic factor through the use of currently available D1R detection methods. Fenoldopam, which is FDA-approved to treat renal hypertension, could be repurposed as an effective therapeutic agent for patients with D1R-expressing tumors. Several drugs that interfere with the cGMP/PKG pathway and are approved for treating other diseases should also be considered as potential treatments for BC.

KEY WORDS: dopamine receptors, breast cancer, cell cultures, xenografts, drug therapy, signaling pathways.

I. INTRODUCTION

Dopamine (DA) is a catecholamine that acts as a major neurotransmitter in the brain and as a circulating hormone in the periphery, where it is produced by sympathetic nerves, adrenal medulla, GI tract, and multiple non-neuronal cells.[1] DA receptors (DAR) are expressed in almost all peripheral organs, and their dysregulation is associated with hypertension, gut motility disorders, metabolic dysfunctions, fertility issues, and cancer. There is significant evidence that DA is involved in both benign and malignant tumorigenesis along the pituitary–gonadal axis and reproductive organs in both males and females.[2]

After discovering expression of functional DAR in human adipocytes and breast adipose tissue,[3] we questioned whether they are also expressed in breast cancer (BC), and if so, what are their functions. The objective of this review is to summarize recent evidence on overexpression of DAR type 1 (D1R) in a significant number of aggressive BC, and its involvement with tumorigenesis. It is well recognized that concepts associated with DA and its receptors are more familiar to neurologists and psychiatrists than to oncologists. Therefore, the first sections of this review cover some of the basic features of dopamine homeostasis, the structure, signaling and functions of its receptors, and the multitude of DAR agonists and antagonists available as therapeutics. The overall goal of this review is to bring D1R to the forefront of critical receptors that can be exploited for the diagnosis, prognosis, and treatment of BC.

II. DOPAMINE HOMEOSTASIS

Homeostasis is a self-regulating process by which biological systems maintain stability while adjusting to changes in internal and external environment. DA homeostasis is governed by interrelated dynamic processes that include synthesis, metabolism, storage, release, and reuptake. These differ in

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several respects between the "closed" system of the brain dopaminergic neurons, and the "open-ended" peripheral dopaminergic system (Fig. 1). In the closed configuration of neuron/synapse/neuron, the concentration of the released DA within the confines of the small space of the synaptic cleft can be as high as 5–10 μM. On the other hand, peripheral DA-producing cells are often remote from their target cells, and the concentration of circulating DA reaching them does not exceed 20–30 nM. The disparity in DA concentrations at brain and peripheral sites should be considered upon evaluating *in vitro* studies with peripheral cells, many of which unfortunately have used DA at high micromolar concentrations.

A. Synthesis, Release, Storage, and Metabolism of Dopamine

The three catecholamines—DA, norepinephrine (NE), and epinephrine (Epi)—are synthesized by four sequential enzymes. The first is tyrosine hydroxylase (TH), which converts tyrosine to dihydroxyphenylalanine (L-Dopa), and serves as the rate limiting step. The second enzyme, Dopa decarboxylase (DDC), generates DA from L-Dopa. Cells that express dopamine β-hydroxylase (DBH) can synthesize NE as a final product. DBH is also secreted into the circulation. The last enzyme, phenylethanolamine N-methyl-transferase (PNMT), generates Epi, and is primarily expressed in the adrenal gland. Metabolic degradation is carried out by two major enzymes: monoamine oxidase (MAO), which carries out oxidative deamination, and catechol-O-methyl transferase (COMT), which carries out O-methylation. Some of the catecholamines can also undergo conjugation by glucuronidases and sulfation by sulfotransferases.

DA is stored within the producing cells in secretory vesicles, which protect it from degradation and enable its regulated release by a calcium-dependent

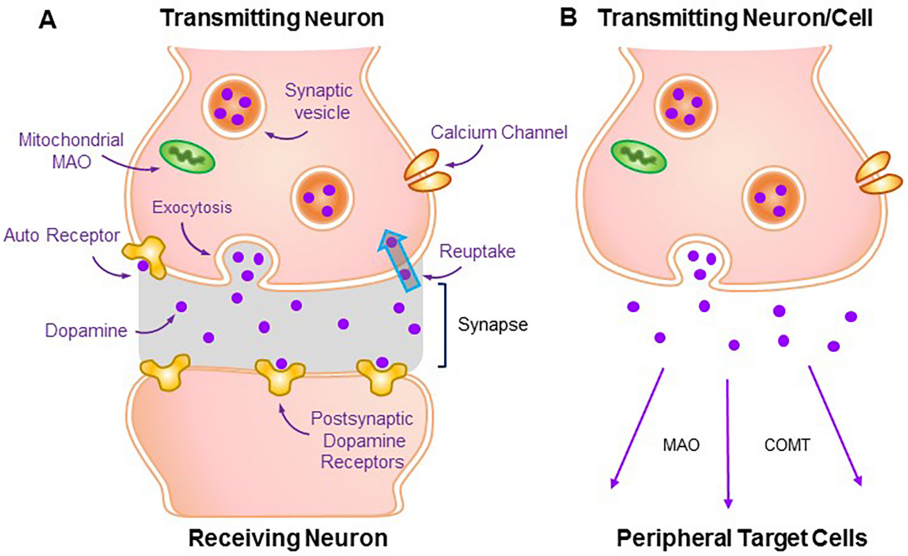

FIG. 1: A model of the "closed" or "open-ended" dopaminergic systems. (A) Most dopaminergic neurons in the brain operate as a "closed system," whereby a transmitting neuron is associated with a receiving neuron through a small gap (the synapse). DA, released by exocytosis, can bind to presynaptic (auto receptors) or postsynaptic receptors and activate various signaling cascades. The actions of DA are terminated by its reuptake into the transmitting neuron. Inside the neuron, DA is either stored in synaptic vesicles or is degraded by mitochondrial monoamine oxidase (MAO). (B) In the "open-ended" system, the released DA is distributed to remote targets by the circulation. In this system, there is no reuptake mechanism and no autoreceptors on the transmitting neuron/cell. Peripheral DA is degraded by MAO and catechol-O-methyl transferase (COMT).

exocytosis.[4] Several transporters—the dopamine transporter (DAT), the vesicular monoamine transporters (VMATs), and the organic cation transporters (OCT)—terminate the action of released DA through reuptake into the secreting cells and its repackaging into the secretory vesicles. This process is especially important within the "closed system" of the brain, and is less operative in the open-ended system where DA diffuses away from the producing cells.

B. The Origins of Circulating Dopamine

The blood–brain barrier prevents a bidirectional transport of DA between the brain and the systemic circulation, while the DA precursor, L-Dopa, easily interchanges between the two compartments. As shown in Fig. 2, circulating DA originates from sympathetic nerves, somatic cells, and dietary sources.[5] The major source of plasma DA is the gastrointestinal (GI) tract, with additional contributions coming from a coincidental release of DA with NE/Epi and DBH from the sympathoadrenal system. Some tissues, which are populated by DA-producing cells, called amine precursor uptake and decarboxylation (APUD) cells,[2] also release DA into the circulation. In addition to *de novo* synthesis of DA from tyrosine, tissues can take up L-Dopa from the circulation via carrier-mediated transport, and converts it to DA by the ubiquitously expressed DDC. Beyond the endogenous sources, DA can be obtained directly from the diet,[6] as certain foods, e.g., bananas, contain DA, while others, e.g., tuna and cereals, contain Dopa. Some foods contain tyramine, which can be converted to DA by the cytochrome P450 enzyme complex (CYP2D6).

In addition to the GI tract, the following organs/cells express TH and/or DDC and can synthesize DA: anterior and posterior pituitaries, kidneys, salivary glands, spleen and pancreas, mesenteric lymph nodes, thymus and lymphocytes, mast cells, bone marrow–derived mesenchymal stem cells, carotid body, keratinocytes, melanocytes, adipocytes, as well as the gonads and most reproductive tract organs.[1] Support for some non-neuronal origin of DA comes from studies

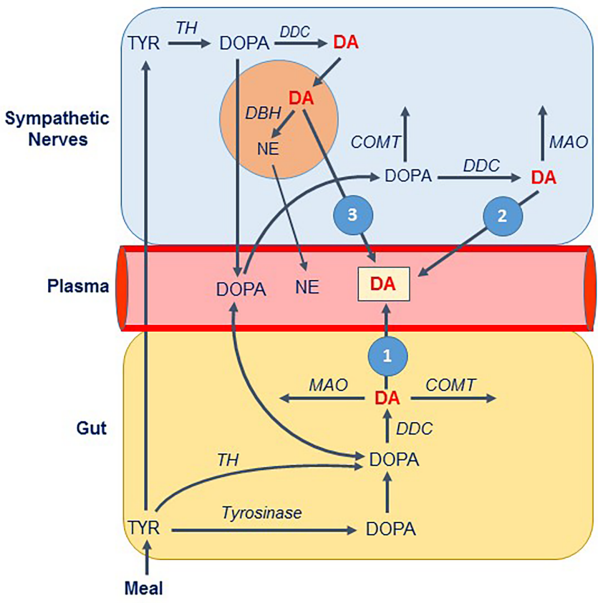

FIG. 2: The various sources of plasma DA. (1) From the gut. (2) From *de novo* synthesis in sympathetic nerves. (3) From conversion of dihydroxyphenyl alanine (DOPA) in sympathetic nerves and other cell types. Sympathetic nerves expressing tyrosine hydroxylase (TH) can generate DA from tyrosine (TYR), available from the diet, or from DOPA, available from both diet and blood. In the gut, either TH or tyrosinase can convert TYR to DOPA. DDC: DOPA decarboxylase; DBH: dopamine beta hydroxylase; MAO: monoamine oxidase; COMT: catecholamine O-methyl transferase.

using chemical sympathectomy in rats, which caused a marked decrease in DA in some organs, but its preservation in others.[7] Only a small fraction of the DA produced in the above sites reaches the circulation, as most DA remains locally and acts as a paracrine/autocrine factor on adjacent DAR-expressing cells.

Except for few reports on plasma DA levels under various physiological and pathological conditions, the presence of DA in the systemic circulation has been ignored in most clinical studies. Analysis of urinary DA and its metabolites, which has been used for some diagnostic purposes, has several drawbacks, including the inability to determine the sources of DA or to assess rapid changes in its release in response to stimuli or medications.

C. Dopamine Sulfate (DA-S): Occurrence and Functions

Unlike the situation in most species, circulating DA in humans is primarily present in the form of DA sulfate (DA-S). Plasma DA in humans is more than 95% sulfonated, with the 3-O-sulfate isomer more abundant by an order of magnitude than the 4-O-sulfate isomers.[5] NE and Epi are also sulfoconjugated, but to a much lesser extent than DA. Under basal conditions, serum DA-S at ≈ 5 ng/ml exceeds by 10-fold the basal levels of free DA (0.3 ng/ml), NE (0.2 ng/ml) or EPI (0.05 ng/ml) combined. Yet, the presence of DA-S in human serum has been mostly overlooked, likely because its detection requires a special extraction method. The sulfation reaction is carried out by sulfotransferase type 1A3 (SULT1A3).[8] Sulfoconjugation, which also affects steroid and thyroid hormones, some neuroendocrine peptides, and glycoprotein hormones, can alter the bioactivity, metabolic half-life, solubility, and/or receptor binding affinity of these hormones.

Ingestion of a meal after fasting in human volunteers induced a 50-fold rise in serum DA-S levels.[5] This and other data revealed that serum DA-S is derived from sulfoconjugation of DA synthesized from L-Dopa in the GI tract, with both dietary and endogenous sources contributing to its circulating levels. A 5–10-fold rise in serum DA-S levels was seen in chronic alcoholics and cocaine abusers, although neither the causes for these rises nor the clinical implications of DA-S to these conditions are known. Serum DA-S levels are also high in patients with renal hypertension, pheochromocytoma, and in those with chronic renal insufficiency.

DA-S does not bind DAR and is biologically inactive. However, unlike the irreversible inactivation of DA by deamination, O-methylation, or glucuronidation, sulfoconjugation is reversible, and DA-S can be converted back to bioactive DA by arylsulfatase A (ARSA), a secretable enzyme which is expressed by several peripheral tissues (Fig. 3). In fact, DA-S has a serum half-life of 4–5 hrs, as compared with 2–3 min for free DA. Thus, DA-S constitutes a relatively stable serum reservoir of inactive (non-degradable) DA, which can be converted back to bioactive DA when needed by cells that express

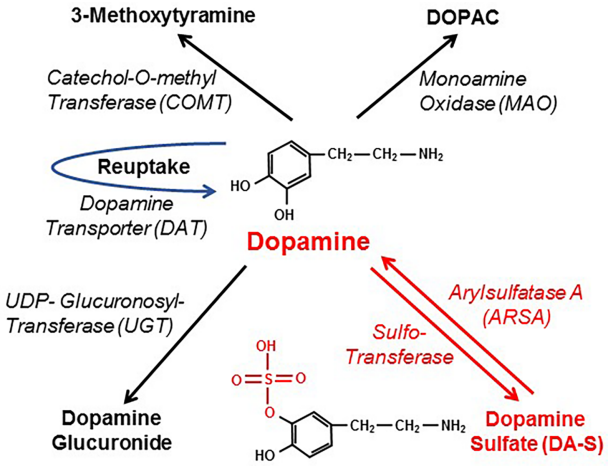

FIG. 3: Dopamine metabolism. DA can be converted to 3-methoxytyramine by catechol-O-methyl transferase (COMT), or to dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase (MAO). DA conjugation to glucuronide is done by UDP-glucuronosyltransferase (UGT), while sulfoconjugation is done by sulfotransferase. The latter reaction is reversible, and DA-S can be converted back to bioactive DA by arylsulfatase A (ARSA).

ARSA. The ability of some normal and malignant tissues to convert DA-S to bioactive DA is fundamental for the understanding of the functions of peripheral DA.

III. DOPAMINE RECEPTORS (DAR) AND THEIR SIGNALING

A. Structure, Classification, and Spectrum of Functions of DAR

The DAR belong to the superfamily of G-protein coupled receptors (GPCRs), which mediate signal transduction via heterotrimeric G-proteins.[9] The GPCR are characterized by a seven trans-membrane α-helical configuration, predicted to form three extracellular and three intracellular loops. The helices are arranged in a circular orientation within the membrane plane, with a crevice in the middle of the helices that contains the ligand binding site. Binding of an agonist induces conformational changes in the cytoplasmic face of the receptor, enabling its linkage to G-proteins and their activation. Activated receptors can also become associated with adaptor

proteins such as G-protein coupled receptor kinases (GRKs) and β-arrestins, which affect receptor signaling and trafficking. Ligand-induced changes in the receptors are followed by activation of several downstream signaling cascades, and are also accompanied by receptor desensitization, internalization, and degradation.[10]

The five DAR are grouped by structure, pharmacology, and function into D1R-like (D1R and D5R) and D2R-like (D2R, D3R and D4R) receptors (Fig. 4). The D1R-like have shorter third intracellular loops (which couple to G-proteins), and longer c-terminal tails (which contain site of phosphorylation and palmitoylation) than the D2R-like. According to the original DAR classification, the D1R-like are coupled to Gαs proteins, activate adenylate cyclase (AC), increase cAMP, and stimulate protein kinase A (PKA), while the D2R-like are coupled to Gαi/o proteins, inhibit AC, suppress cAMP, and inhibit PKA.[9] However, this older classification is oversimplified, since the DAR can couple to other G-proteins and activate alternative pathways such as the guanylate cyclase (GC)/cGMP/protein kinase G (PKG) pathway,[11] as discussed in great details below.

The concept of DAR oligomerization was initially met with skepticism, but biophysical approaches such as fluorescent resonance energy transfer (FRET), bioluminescent resonance energy transfer (BRET) and atomic force microscopy (AFM), have established that DAR heterodimerization is a prevalent phenomenon with substantial physiological, pathological, and therapeutic implications.[12] The DARs can form four types of oligomers: (1) homodimers, e.g., D1R-D1R; (2) heterodimers with another member of the DAR subfamily, e.g., D1R-D2R; (3) heterodimers with another GPCR, e.g., D1R-A1R (adenosine A1 receptor), and (4) heterodimers with a structurally different receptor, e.g., DAR-$GABA_A$ (receptor for gamma aminobutyric acid), or DAR/SSTR1 (somatostatin receptor 1). As most of these receptors are expressed in the same organs that express DAR, heterodimers have the potential to influence both the functions and therapeutic responses of peripheral DA.

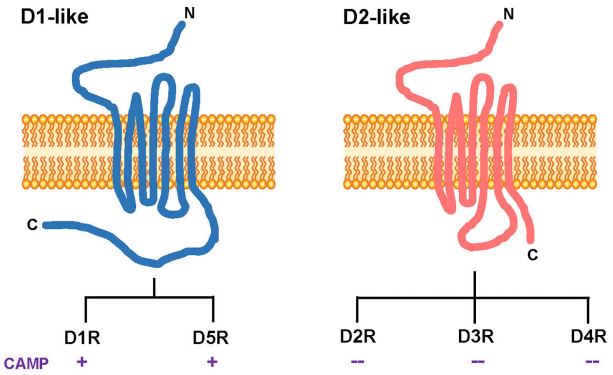

FIG. 4: Comparison of the structure and second messengers of the DAR. D1-like have a longer c terminal tail and a smaller third intracellular loop that links to G-protein than the D2-like. The D1-like are generally associated with increased cAMP via activation of Gs, while the D2-like are primarily associated with inhibition of cAMP via Gi.

The DAR have a very broad spectrum of neurologic, psychologic, endocrine, renal, cardiovascular, metabolic, immune and oncogenic actions.[1] A search in the PubMed data base for the term "dopamine receptors" in the title or abstract, brings up more than 15,000 entries, underscoring the all-encompassing physiological and pathological significance of these receptors. Within the brain, the DAR regulate voluntary movements, reward, pattern of sleep, working memory, feeding, attention, cognitive functions, olfaction, vision, and reproductive behavior. In the periphery, the DAR are involved in some aspects of the operation of the sympathetic nervous system (SNS), and participate, often independent of the SNS, in the control of cardiovascular, renal, gastrointestinal, immune, and reproductive functions. The DAR are also associated with malignant transformation of several tissues and organs. As discussed below, certain DAR are expressed by all components that compose the human breast: ducto-lobular units, adipocytes, endothelial cells, and infiltrating immune cells.

B. Canonical Signaling by the DAR

AC plays a key role in mediating the actions of DAR. It catalyzes the conversion of ATP to cAMP, a second messenger that controls cAMP-dependent kinases, a variety of transcription factors, and

many ion transporters. Increased cAMP levels activate PKA, which acts upon targets such as CREB (cAMP response element binding protein) and DARPP-32 (32-kDa dopamine and cAMP-regulated phosphoprotein). Binding of four cAMP molecules to the R subunit of PKA promotes certain conformational changes resulting in dissociation and activation of the C monomers. These, in turns, phosphorylate cytoplasmic and nuclear proteins that have the appropriate consensus sequence. CREB is a transcription factor which is activated by PKA-induced Ser133 phosphorylation, and regulates transcription of numerous target genes.[13]

C. DARPP: A Dopamine Regulated Phosphatase Involved in Tumorigenesis

DARPP-32 (also named Dp32) was discovered as a 32 kDa protein substrate of DA-activated PKA in the brain.[14,15] As shown in Fig. 5A, Dp32 mediates downstream signaling of DA via D1R, and is negatively regulated by DA via D2R, as well as by glutamate through the N-methyl-D-aspartate receptor (NMDAR). Phosphorylation of Thr-34 of Dp32 by PKA converts it into a potent inhibitor of protein phosphatase 1 (PP1), which controls the activity of hundreds of phosphorylated proteins. Phosphorylation of Dp32 at Thr-75, transforms Dp32 into an inhibitor of PKA, a protein whose phosphorylation leads to cell survival and blockade of apoptosis. Hence, Dp32 acts as a tightly regulated hub molecule which mediates many actions of DA. Dp32 activity was thought to be limited to neurons until 2002, when a truncated transcript was discovered in gastric cancer cells. This isoform, named tDp, codes for an N-terminal-truncated isoform that lacks the first 36 residues.[14] Since then, both tDp and Dp32 were found to be over-expressed in breast, colon, esophageal, gastric, lung and prostate cancers, where they exert oncogenic actions (Fig. 5B).

D. Non-Canonical Signaling of DAR: The cGMP-Protein Kinase G Pathway

The cGMP pathway, shown in Fig. 6, deserves a special attention, as it is the major pathway that is activated by D1R in breast cancer.[16] Two guanylate cyclases—particulate (pGC) and soluble (sGC)—generate cGMP from GTP. The pGCs are activated by natriuretic peptides, while cytosolic sGCs are the main targets of nitric oxide (NO), and can be stimulated by two drugs: YC-1 and riociguat.[17] Elevated cGMP activates protein kinase G (PKG), which acts upon a number of targets, including DARPP.[18] Once elevated, both cAMP and cGMP are rapidly hydrolyzed by phosphodiesterases (PDEs), an 11-member superfamily that differ in substrate specificity and catalytic properties.[19] Viagra (sildenafil), Cialis (tadalafil), and Levitra (vardenafil), which are used to treat erectile dysfunction, selectively inhibit PDE5 which hydrolyzes cGMP.[20] This results in a sustained cGMP rise, which stimulates penile erection in the male genitalia, and induces apoptosis in multiple carcinomas.[21]

The above characteristics underscore the difficulty in predicting the actions of DA at any given target. This uncertainty results from the following: (1) DA can activate five different receptors that often act in opposite directions (hence, the ratio of the different DAR expressed in a given cell affects its overall response to DA): (2) DARs can homo- or hetero-dimerize and become linked to a variety of G-proteins that activate a multitude of signaling pathways; (3) the responses to DA can be altered by GRKs, which also have receptor-independent actions; and (4) the response to receptor activation is dynamic and can change because of time-dependent receptor desensitization.

IV. DOPAMINERGIC DRUGS

A. Overview of DAR-Selective Ligands

The well-recognized association of brain DA with many neurological conditions has fostered a vast investment by the pharmaceutical industry in the development of DA altering drugs. Drugs that target DAR comprise one of the largest classes of pharmaceuticals. They have many therapeutic applications for brain-associated disorders, as well as for selected endocrine, cardiovascular and renal disorders.[1] DAR-targeting drugs

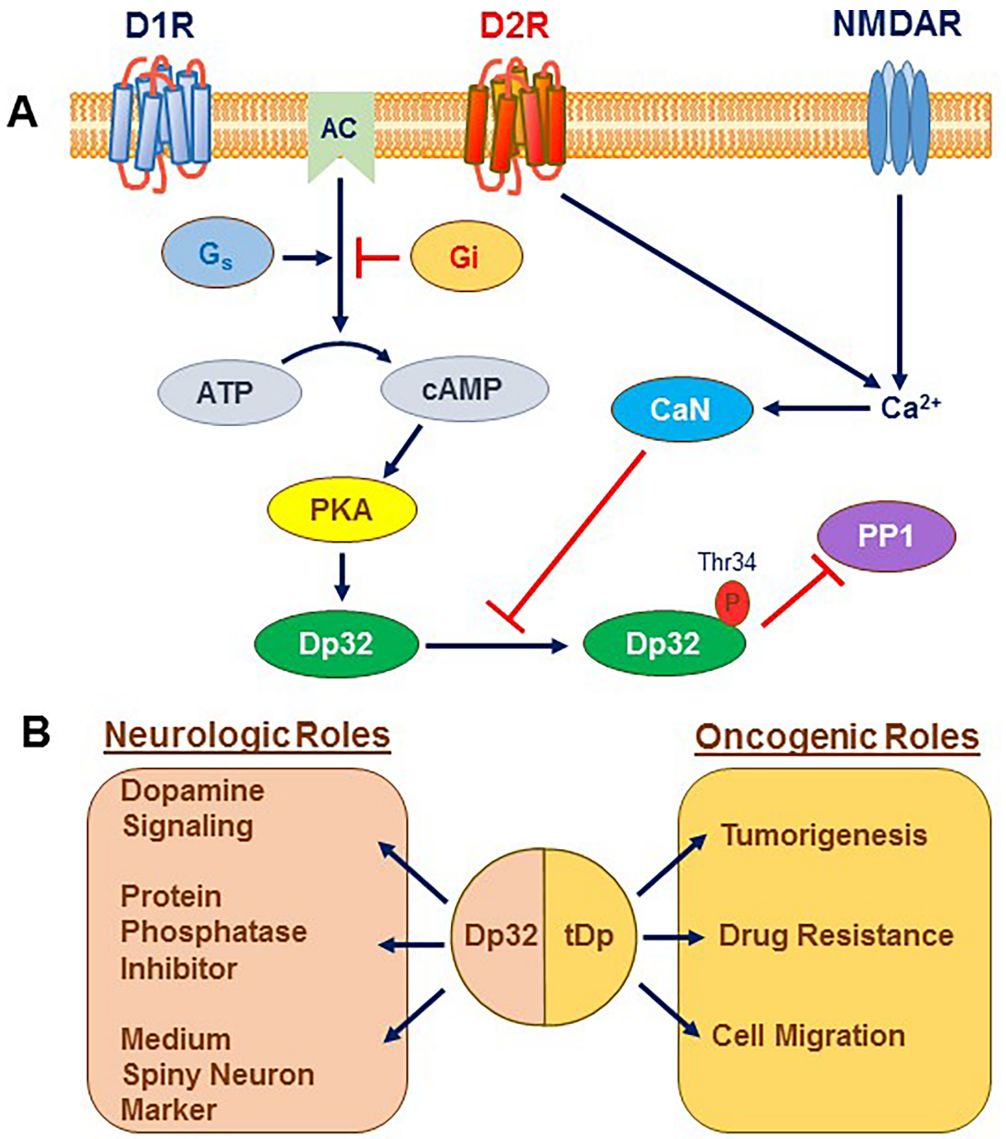

FIG. 5: DARPP characteristics. (A) The dopamine and glutamate signaling pathways that modulate the phosphatase 1 inhibitory activity of DARPP in neurons. AC, adenylyl cyclase; CaN: calcineurin; Dp32, DARPP-32; NMDAR: N-methyl-D-aspartate receptor; PP1, protein phosphatase 1; Thr34-site of phosphorylation on Dp32. (B) The multiple neurologic and oncogenic actions of Dp32 and truncated DARPP (tDp). Adapted and modified from Avanes et al.[15]

can be divided into the following categories: (1) therapeutic drugs for treating neurological disorders; (2) drugs that do not penetrate the brain, for treating peripheral DA-related disorders; (3) drugs used in basic research as receptor probes or radioligands; and (4) diagnostic drugs for medical imaging. In addition to those drugs that bind DAR, many others affect DA synthesis, reuptake, or metabolism,[22] but will not be further discussed here.

B. Selectivity and Applications of DAR Agonists and Antagonists

To cover the full range of drug actions at the DAR, an advanced drug definition was proposed

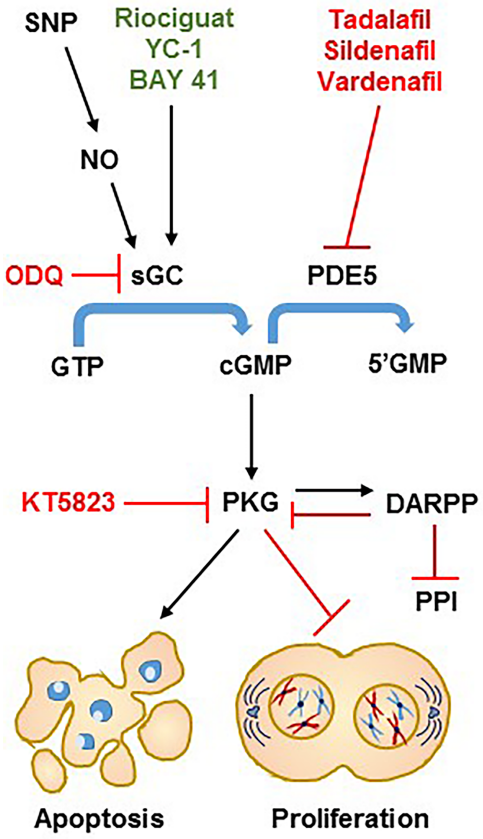

FIG. 6: The guanylate cyclase/cGMP/PKG pathway. Soluble guanylate cyclase (sGC) is activated by nitric oxide (NO), and by three drugs: riociguat, YC-1 and Bay 41-2271 (BAY 41) and is inhibited by ODQ. Activated sGC converts GTP to cGMP while phosphodiesterase 5 (PDE5) selectively hydrolyzes cGMP to 5'GMP. Inhibitors of PDE5 include tadalafil (Cialis), sildenafil (Viagra) and vardenafil (Levitra). Elevated cGMP activates protein kinase G (PKG), which is inhibited by KT5823 and in some systems can induce apoptosis and/or inhibit cell proliferation. SNP: sodium nitroprusside, which often serves as a NO donor.

in 2008.[23] According to this concept, an agonist is defined as a substance that binds to a specific receptor and stimulates the signaling pathway known to be associated with it. A partial agonist causes less than maximal response, but can also act as a partial antagonist. An antagonist has no effects on its own but blocks an agonist-induced signaling. An inverse agonist, a somewhat confusing term, defines a ligand that binds to a receptor and inhibits agonist-independent (constitutive) signaling.

Drugs that target D2R constitute the largest category of therapeutic dopaminergic agents. D2R agonists are used to treat Parkinson's disease, drug abuse, sexual dysfunction, hyperprolactinemia, and restless leg syndrome, while D2R antagonists are used to treat various types of psychosis. The DA hypothesis of schizophrenia stipulated that positive symptoms of the disease, i.e., disorganized thoughts, delusion, and hallucination, are caused by hyperactivity of D2R neurotransmission in the subcortical and limbic brain regions.[24]

The serendipitous discovery that D2R antagonists such as chloropromazine and haloperidol ameliorated some symptoms of psychosis, ushered an era of neuroleptic ("tranquilizing") drug discovery. However, it was soon realized that such drugs, later renamed "first generation antipsychotics," caused severe side effects such as Parkinsonism, tardive dyskinesia, and elevated serum prolactin (PRL) levels, all of which are typical of D2R blockade. These shortcomings provided strong incentives to search for drugs with lesser extrapyramidal side effects, leading to the development of the second generation or "atypical antipsychotics" (AAP) drugs.[25] AAP are prescribed for many mental disorders: schizophrenia, bipolar disorder, mania, attention deficit disorder, major depression, posttraumatic stress disorder, and autism. All AAP antagonize D2R (and other DAR with lesser affinity), but also bind at variable affinities to a myriad of serotonergic, adrenergic, muscarinic, and histaminergic receptors.[26] Their clinical effects, therefore, cannot be clearly assigned to their action on a specific receptor.

D1R is the most highly expressed DAR in the brain.[11] Unlike D2R-like which are present at both presynaptic and postsynaptic localizations, D1R and D5R are found only postsynaptically and do not function as autoreceptors. The importance of D1R for brain functions is highlighted by the finding that of all the individual DAR knockouts in mice, the D1R knockout has the most severe phenotype, including spatial learning deficits, hyperactivity, and abnormal memory retention.[27] Given the high

homology in the binding pocket of D1R and D5R, presently available drugs do not discriminate well between the two receptors.

C. Peripheral Dopaminergic Altering Drugs

Typical brain capillaries differ from most peripheral capillaries by having tight junctions between the endothelial cells lining the vessel's lumen, thus forming the blood–brain barrier (BBB). Brain regions that lack the BBB include the area postrema, median eminence of the hypothalamus, posterior pituitary gland, and pineal gland. The BBB excludes from the brain all large-molecule drugs, and over 98% of small-molecule drugs.[28] Small molecules with a molecular mass of less than 400–500 Da, which also form less than 8–10 hydrogen bonds with solvent water, can cross the BBB in significant amounts. Almost all the clinically useful DAR altering drugs penetrate the brain, and only few, classified as peripheral drugs, do not.

Two peripheral D2R antagonists are domperidone[29] and metoclopramide.[30] Both are prokinetic drugs that increase GI motility and have been used in clinical practice to treat several peripheral DA-associated disorders. Metoclopramide binds to D2R at nanomolar affinity, has lower binding affinity to D1R, and also acts as a mixed serotonergic antagonist/agonist. Metoclopramide is commonly used to treat nausea and vomiting associated with chemotherapy.

Fenoldopam is a peripheral D1R agonist with a weak antagonistic activity at alpha-1 and alpha-2 adrenergic receptors. It acts as a vasodilator in the peripheral arteries, and as a diuretic in the kidneys.[31] Fenoldopam has been approved by the FDA in 1997 for the short-term management of severe hypertension, when a rapid reduction of blood pressure is required.[32] In hypertensive patients, Fenoldopam rapidly decreased blood pressure, increased renal blood flow, and maintained or improved the glomerular filtration rate. In normotensive volunteers, fenoldopam increased renal blood flow without affecting systemic blood pressure or heart rate. As discussed below, we reported[33] that fenoldopam, acting via the D1R/sGC/cGMP pathway, was a potent inducer of apoptosis in particularly aggressive breast cancer cells, and a robust suppressor of BC xenografts in mice.

V. PREVALENCE, CLASSIFICATION, AND TREATMENTS OF BREAST CANCER

Tumors develop because of malfunctions of genes that regulate cell growth or cell death. Some mutations can lead to rapid, unchecked growth, creating tumors that expand quickly and damage nearby tissues. Such tumors can produce enzymes that dissolve the surrounding tissue and grow beyond their normal boundaries, a process defined as invasiveness. Many tumor cells can also be released into the blood stream, invade remote organs, and grow at distant locations, a process defined as metastasis.

A. Epidemiology and Molecular Characterization of Breast Cancer

BC is the most common malignancy among women, with more than one million cases occurring worldwide each year. Although rare, men can also get BC, with about 1 out of every 100 diagnosed cases found in a man. In women, increased risk of developing BC correlates with early menarche, nulliparity, late age at first childbirth, and late menopause, as well as with obesity and exposure to exogenous hormones. Mutations in *BRCA1*, *BRCA2*, and *ATM* genes are associated with increased risk of BC, although heritable BC accounts for no more than 10–15% of all cases.[34] BC primarily arises in the terminal ductal-lobular units, and is classified by different criteria, based on tumor size, histologic grade, lymph node status, proliferation index, and status of hormone receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).

As depicted in Fig. 7, four molecular BC subtypes are recognized:

1. Luminal A is ER and PR positive and HER2 negative, with a low proliferation index. This is the most common BC subtype, occurring in about half of the patients. Such tumors grow slowly and have the best prognosis.

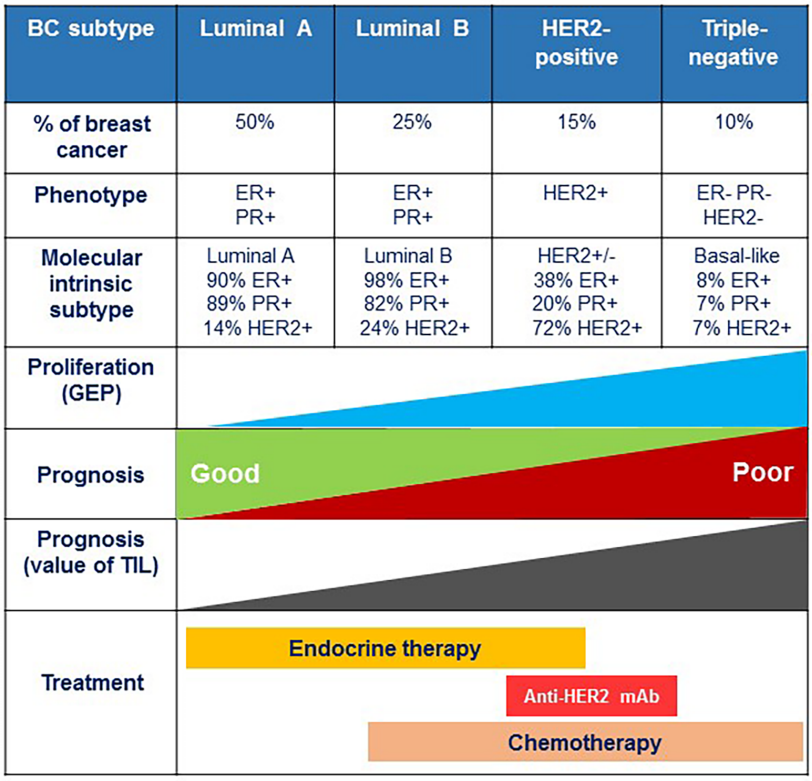

FIG. 7: Classification, prognosis and treatment of breast cancer based on hormone receptor expressions. GEP: gene expression profiling; TIL: tumor infiltrating lymphocytes. Adapted and modified from http://www.pathophys.org/breast-cancer/.

2. Luminal B is hormone-receptor positive, HER2 positive or negative, and high proliferation index. Such tumors occur in ~ 25% of cases, grow slightly faster than luminal A, and their prognosis is slightly worse.
3. HER2-positive is hormone-receptor negative and HER2 enriched. These tumors grow faster than luminal cancers, and have a poor prognosis.
4. Basal-like, triple-negative is hormone-receptor negative and HER2 negative. This tumor subtype is more common in women with *BRCA1* gene mutations, and in younger women. Most triple-negative BCs are aggressive with poor prognosis.

Additional refinements of BC classification[35] include the status of claudins, transmembrane proteins that are enriched in tight junctions and are involved in cell migration and epithelial mesenchymal transition (EMT).

B. Surgery and Chemotherapy in Breast Cancer

Tumors that are aggressive and life-threatening must be managed robustly, i.e., by a combination of surgery, radiotherapy, and chemotherapy. The three types of surgeries are (1) lumpectomy, or removal of the tumor along with a margin of surrounding tissue; (2) quadrantectomy, or removal of the tumor with a larger area of nearby tissue; and (3) mastectomy, or removal of the entire breast on one or both sides. Depending upon the biopsy results, axillary lymph nodes may be removed. Radiotherapy uses controlled doses of radiation, and is usually given after surgery and chemotherapy to kill any remaining cancer cells.

Neoadjuvant chemotherapy is used to reduce tumor size before surgery, while adjuvant chemotherapy is used after tumor excision. Chemotherapy is the mainstay treatment for patients with triple negative tumors that are resistant to hormone or targeted therapy, and for those with advanced metastatic disease.[36] Over the years, dozens of anticancer drugs have been developed, with the treatment options taking into account tumor grade and histology, and whether the desired outcome is curative or palliative. Most regimens combine drugs that act via different mechanisms in order to improve the odds of suppressing tumor growth.

The most common classes of chemotherapeutic drugs are (1) anthracyclines such as doxorubicin, which stop DNA replication and induce apoptosis; (2) anti-mitotic agents such as paclitaxel, which stabilize microtubules and prevent cell division; (3) anti-metabolites such as 5-fluorouracil, which stop DNA synthesis by blocking thymidylate synthase; (4) alkylating agents such as cyclophosphamide, which form DNA crosslinking, leading to apoptosis; and (5) platinum agents such as cisplatin, which form DNA adducts that induce cell cycle arrest. Chemotherapy can cause various side effects, depending upon the type and dose of the drugs given, and length of treatment. In younger women, changes in the menstrual periods are a common side effect of chemotherapy. Premature menopause and infertility also occur, with increased risk of bone loss and osteoporosis.

C. Immunotherapy in Breast Cancer

Evasion of antitumor surveillance by the immune system is a hallmark of the emergence and progression of cancer.[37] Tumors employ multiple mechanisms to avoid recognition by the immune system, including expression of the T cell regulatory molecule PD-L1 (programmed death ligand 1). The PD-1 receptor and ligands PD-L1 and PD-L2 play critical roles in T cell co-inhibition.[38] Overexpression of PD-L1 and PD-1 on tumor cells and tumor-infiltrating lymphocytes, respectively, correlates with poor outcome in several cancers. Monoclonal antibodies (mAbs) that block the PD-1/PD-L1 pathway have shown impressive responses in melanoma, non-small-cell lung cancer, renal cell carcinoma, and bladder cancer. In 2019, the FDA approved use of atezolizumab, an anti-PD-L1 mAb, to treat triple-negative, metastatic BC that express the PD-L1 protein. In 2021, the FDA approved another immunotherapeutic drug, pembrolizumab (Keytruda), for early-stage, triple-negative BC in combination with chemotherapy as neoadjuvant treatment, and then continued as a single agent as adjuvant treatment. Given the relatively limited T-cell infiltration of most BC, development of strategies enabling sufficient lymphocyte infiltration in BC, and generation of *de novo* T-cell responses, appear to be keys to success of such immunotherapy in BC patients.[37]

D. Hormone and Targeted Therapies in Breast Cancer

Endocrine therapy is primarily aimed at suppressing the oncogenic actions of estrogens. Humans have two ERs: ER alpha (ERα) and ER beta (ERβ), which are encoded by different genes. ERα is expressed at low levels in the normal breast, and its expression increases in carcinomas.[39] Currently effective ER-targeting drugs belong to three classes: (1) selective ER modulators (SERMs) such as tamoxifen, (2) aromatase inhibitors such as anastrozol, and (3) selective ER down-regulators such as fulverstrant.[40] Although there is controversy among scholars, ERβ is generally thought to have anti-proliferative effects in BC progression. The role of the PR in BC has also been controversial. PR expression is considered a surrogate marker for ERα integrity, with high total PR levels correlating with improved tamoxifen response, longer disease-free, and overall survival.

HER2-positive tumors usually have higher tumor grade, tend to grow faster, and are more likely to spread to the lymph nodes. Early stage HER2-positive BC are 2–5 times more likely to recur than HER2-negative tumors. Drugs used to treat HER2-positive tumors belong to two classes: mAbs against the receptor, e.g., trastuzumab, and small molecule inhibitors of receptor signaling, e.g., lapatinib. Over time, however, many treated tumors develop resistance to these therapies, curbing the success of treatment.

Prolactin (PRL) is another hormone that affects BC. In humans, PRL is secreted by the pituitary lactotrophs as well as by many normal and malignant extra pituitary sites, including breast tissue, adipocytes, and lymphocytes. Accumulating evidence demonstrates that both circulating and locally-produced PRL increase BC growth and metastases and confer chemoresistance.[41] Since PRL release is inhibited by DA, acting via D2R,[42] the use of dopaminergic agent to treat BC should take into account their potential effects on PRL.

VI. EXPRESSION OF DAR AND DARPP IN BREAST CANCER

A. Overexpression of D1R in Breast Cancer

Using both RT-PCR and immunocytochemistry, we found overexpression of D1R in a small sample of primary breast tumors, but not in adjacent normal breast tissue.[33] We then compared D1R and D2R expression in eight breast cancer cell lines (BCC) with different tumorigenic properties. D1R was more abundant in aggressive, triple-negative cells than in ER-positive cells (Fig. 8A). All cells also expressed variable amounts of D2R. Cloning of the *DRD1* transcript from MDA-MB-231 BCC confirmed its identity with the published sequence.

Subsequently, we decided to focus on D1R and used tissue microarrays containing 751 breast tumors and 30 normal breast tissue samples to score D1R expression by immunocytochemistry.[33] As summarized in Fig. 9A, strong to intermediate D1R staining was seen in ~ 30% of the tumors, 15% had a weak signal, and the remainder, as well as all normal breast tissue samples, were D1R-negative. Further analysis revealed that D1R staining was

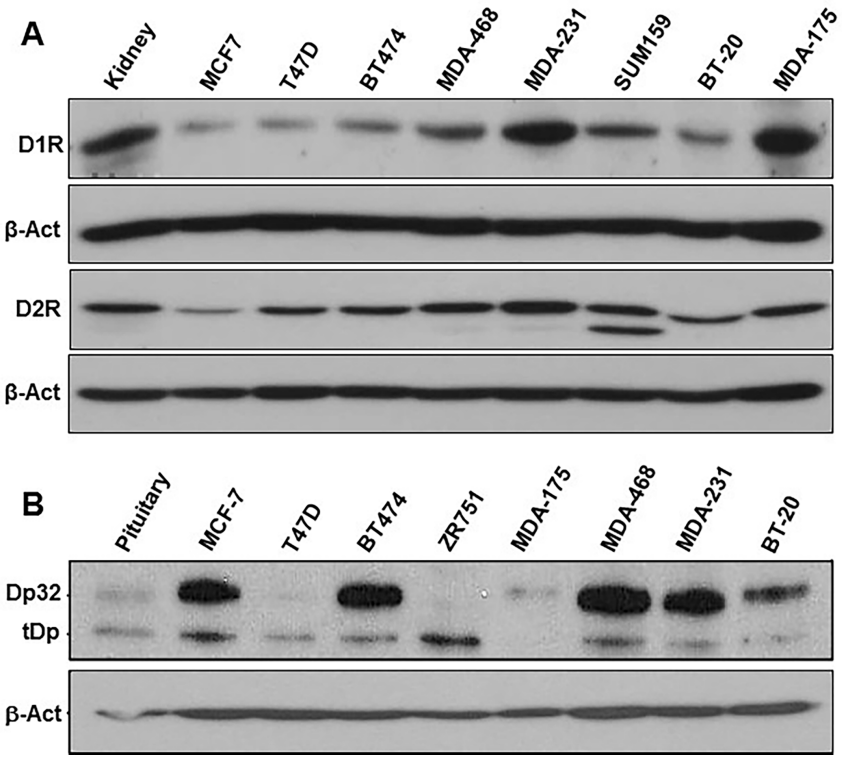

FIG. 8: Expression of DAR and DARPP proteins in breast cancer cell lines. (A) Western blotting of D1R and D2R proteins in 8 breast cancer cell lines; human kidney extract served as a positive control. β-Act: β-Actin. Adapted from Borcherding et al.[33] (B) Western blotting of DARPP (Dp32) and truncated DARPP (tDp) in 8 breast cancer cell lines; human pituitary extract served as a positive control. Unpublished data from the authors' laboratory.

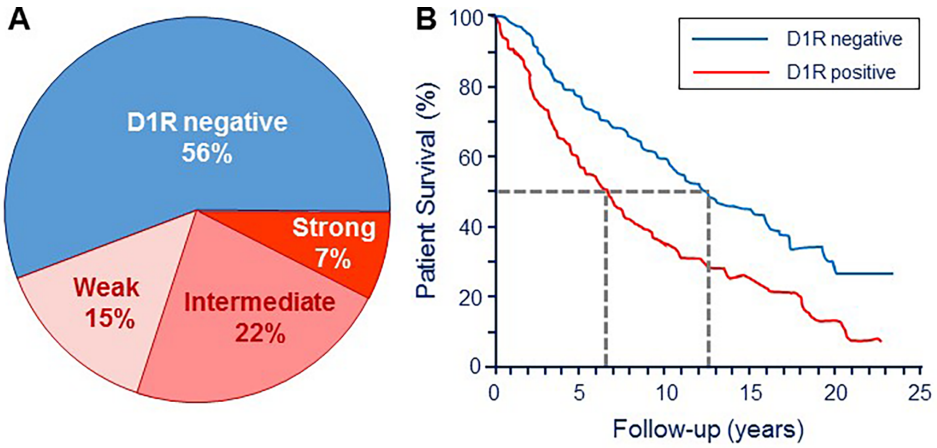

FIG. 9: D1R expression in breast cancer and patient survival. (A) Distribution of immunoreactive D1R in tissue microarrays containing 751 breast carcinomas and 30 normal breast tissue samples. Data shown are the percent of total tumor number. All 30 normal tissue samples were DIR-negative. (B) Positive D1R expression is associated with shorter patient survival, as determined by Kaplan–Meier analysis of 508 tumors.

significantly linked to pre-menopausal age, ER-negative, PR-negative, but HER2-positive tumors, indicating that D1R-overexpressing tumors do not fit within the conventional "triple negative" category, and may constitute a novel high risk category. Indeed, D1R-positive tumors were significantly associated with hallmarks of advanced disease: higher tumor stage, higher tumor grade, and node metastases. Moreover, Kaplan–Meier analysis of 508 tumors revealed that patients with D1R-positive tumors had a significantly shorter median survival time: 6.5 years vs. 12.5 years for those with D1R-negative tumors (Fig. 9B). Recurrence-free survival was similarly shortened.[33] DAR expression in cancer stem cells was previously reported,[43] with more recent studies finding D1R expression in several BCC,[44] and D2R-like expression in BCC[45] and primary breast tumors.[46]

Given that the ER are often not expressed in BC metastases,[47] we questioned whether BC metastases retain D1R expression. Using tissue microarrays with 120 matched breast carcinomas and lymph node metastases, we found an almost identical pattern of D1R expression in both samples (unpublished data). Although these are interesting preliminary data, it is imperative to verify whether systemic BC metastases in liver, lung, or bone, also maintain D1R expression. Others reported higher expression of D2R/D4R in peripheral blood mononuclear cells from BC patients than in healthy people.[48]

B. Expression and Functions of DARPP in Breast Cancer

As summarized in a recent review,[15] many studies have reported that the *PPP1R1B* gene is overexpressed at both the transcript and protein levels in many BC, and documented its involvement in tumorigenesis, based on studies with cultured cells, xenografts, and analysis of human clinical data. In our studies, we used Western blotting to determine expression of Dp32 and tDP proteins in 8 selected BCC. As shown in Fig. 8B, we found overexpression of Dp32 in 5/8 BCC, and overexpression of tDP in 3/8. There was no clear association between overexpression of Dp32 or tDp or the ER/HER2 status in these cells.

Although the overall frequency of tDp/Dp32 expression in primary BC varies among different reports, high expression was consistently correlated with shorter patient survival and drug resistance. Indeed, overexpressed tDp was linked to acquired resistance to trastuzumab[49] and lapatinib[50] in HER2-positive BCC, and to increased cellular proliferation and inhibition of apoptosis.[51] Other data suggested that the Dp32 signaling nexus

plays a role in BC subgroups such as ER-positive disease.

Whereas most studies showed that Dp32 and tDp have the same functions, there are some discrepancies.[15] For example, in one study, tDp, but not Dp32, conferred resistance to trastuzumab in HER2-positive BC by causing AKT phosphorylation and activation of both PKA and CREB, while others reported that Dp32 reversed the effect of tDp on all of these activities. To examine whether tDp and Dp32 alter each other's activities through hetero-oligomerization, crosslinking experiments were conducted, but no significant hetero-oligomerization was detected. It thus appears that the cell context plays a critical role in modulating tDp and Dp32 downstream activities.

VII. ACTIONS OF DOPAMINE AND ITS SIGNALING IN BREAST CANCER AND THERAPEUTIC APPLICATIONS

A. Association of Dopamine with Reproductive Tract Tumors

An involvement of DA in cancers of the reproductive organs was revealed in a large retrospective survey conducted in Sweden in 2013.[52] The survey examined for associations between schizophrenia (representing over-activity of the brain dopaminergic systems), and increased risk of cancer in different organs. Of the 59,233 schizophrenic patients surveyed, 6,137 developed cancer during the 40 years of the survey. The overall cancer incidence among schizophrenic patients and their first-degree relatives was *significantly lower* than that in the general population. This suggested that some genetic factors, presumably contributing to the development of schizophrenia, protect against some cancers. In contrast, female patients had *increased* incidence of breast, cervical, and endometrial cancers, but only after the first diagnosis of schizophrenia. Although not directly tested in this study, the authors speculated that when patients are diagnosed with schizophrenia, most, if not all, are treated with antipsychotics. Thus, the anti-D2R activity of such medications could have been a major reason for the increased cancer incidence in reproductive tissues.

B. Unexpected Actions of Dopamine in Breast Cancer Cells: Activation of the cGMP/PKG Pathway

After discovering overexpression of D1R in aggressive BCC, we explored what could be its putative actions in these cells.[33] Based on data obtained with other overexpressed receptors in BC (ER, EGFR), we fully expected that D1R-agonists stimulate cell growth. Surprisingly, when used at low nM doses (0.1 to 10 nM), DA as well as three D1R-selective agonists—SKF38393 (SKF), A68930 (A6) and fenoldopam (Fen), but not cabergoline (Cab), a D2R agonist— suppressed cell viability (Fig. 10) in 4 triple negative BCC (MDA-MB-231, MDA-MB-468, BT-20 and SUM159), but not in 2 ER-positive cells (T47D and MCF7). We also found that the suppression of cell viability was due to apoptosis. D1R activation also inhibited cell invasion, and increased cell sensitivity to cytotoxicity by doxorubicin.

Since D1R agonists are classified as cAMP activators, the effects of D1R activation on cAMP accumulation was next examined. Unexpectedly, short term cell incubation with DA or fenoldopam increased cGMP rather than cAMP (Fig. 11A). Forskolin (For), a direct AC activator, induced a six-fold increase in cAMP, without having an effect on cGMP, indicating existence of an intact AC/cAMP pathway. Involvement of the cGMP/PKG axis was further verified by the facts that a direct stimulation of sGC by YC-1 (Fig. 11B), and a blockade of PDE5 (which breaks down cGMP) by Cialis (Fig. 11D), augmented cGMP levels, increased PKG activity, suppressed cell viability, and induced apoptosis. Furthermore, KT5823 (KT), a selective PKG inhibitor, prevented fenoldopam-induced apoptosis, confirming mediation of apoptosis by PKG (Fig. 11C), while SCH39166 (SCH), a D1R antagonist, abrogated DA-induced inhibition of cell viability (Fig. 11E). Similar findings were reported in a study by others that cGMP, via PKG activation, suppressed both ER-positive and ER-negative BCC.[53]

C. Suppression of Growth of BC Xenografts in Athymic Nice via D1R Activation

To determine the effects of D1R activation *in vivo*, athymic nude mice were orthotopically implanted into

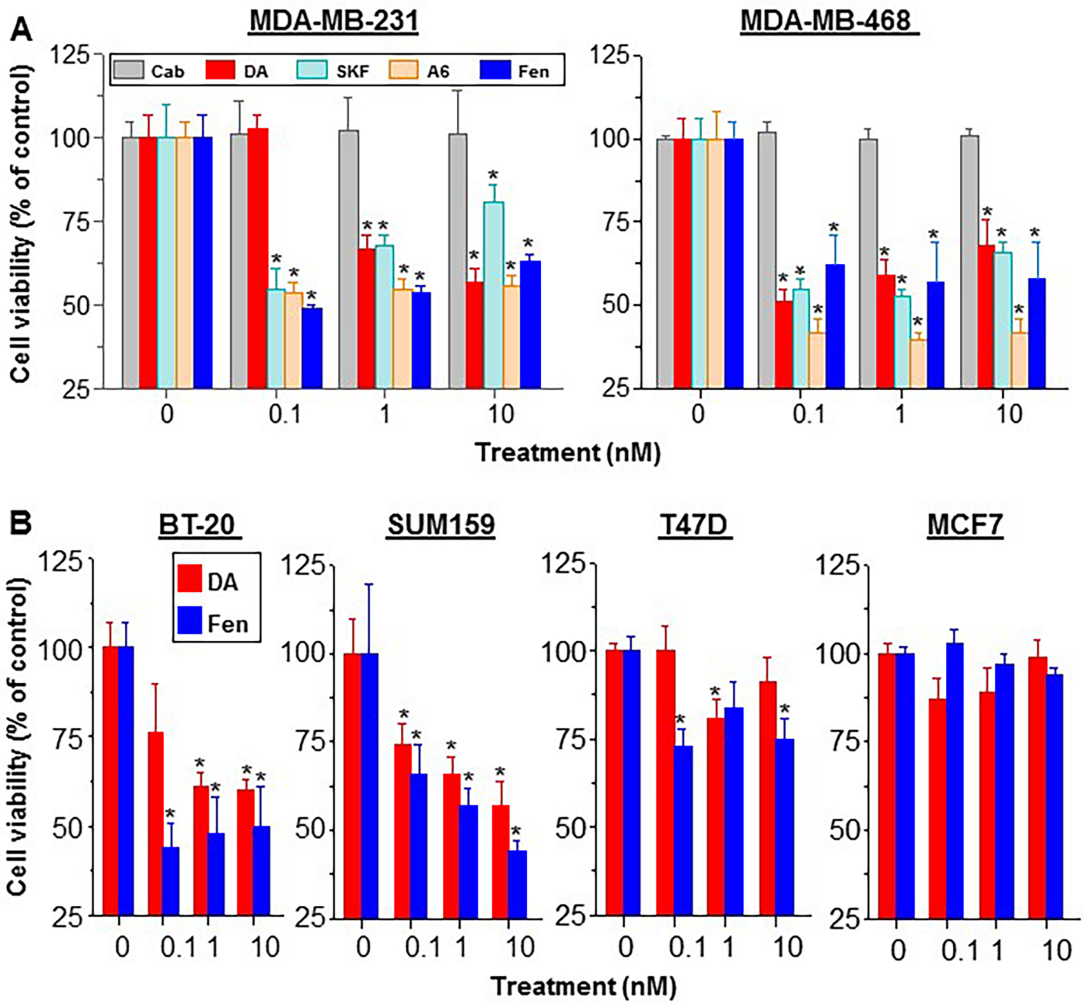

FIG. 10: DA and D1R agonists, but not a D2R agonist, reduced the viability of multiple breast cancer cells. (A) MDA-MB-231 and MDA-MB-468 cells were incubated for 4 days with increasing concentrations of DA, cabergoline (Cab), a D2R agonist, or three D1R agonists: SKF38393 (SKF), A68930 (A6) or fenoldopam (Fen). Cell viability was determined by a resazurin assay. (B), BT-20, SUM159, T47D and MCF7 cells were incubated with increasing concentrations of DA or Fen. *$p < 0.05$. Adapted from Borcherding et al.[33]

the inguinal mammary fat pad with MDA-MB-231 (Fig. 12A). Other mice were implanted in the flank with SUM159 cells. After one week, fenoldopam was continuously delivered via subcutaneously implanted Alzet osmotic mini-pumps. Within several days, the D1R agonist dramatically suppressed tumor growth in the two animal models by increasing both apoptosis and necrosis.[33]

A combination of apoptosis and necrosis likely explains the more robust suppressive effects of fenoldopam *in vivo* than *in vitro*. Increased necrosis could have been due to inhibition of angiogenesis, as DA is known to reduce tumor angiogenesis by inhibiting vascular endothelial growth factor (VEGF) and its receptor via endothelial DAR.[54] Notably, the suppression of tumor growth by fenoldopam was long lasting, as the treated tumors remained quiescent for at least two more weeks after removal of the Alzet pumps (Fig. 12A).

A fluorescent imaging method for visualizing D1R-expressing tumors and metastases was also developed.[33] Figure 12B shows that intravenous

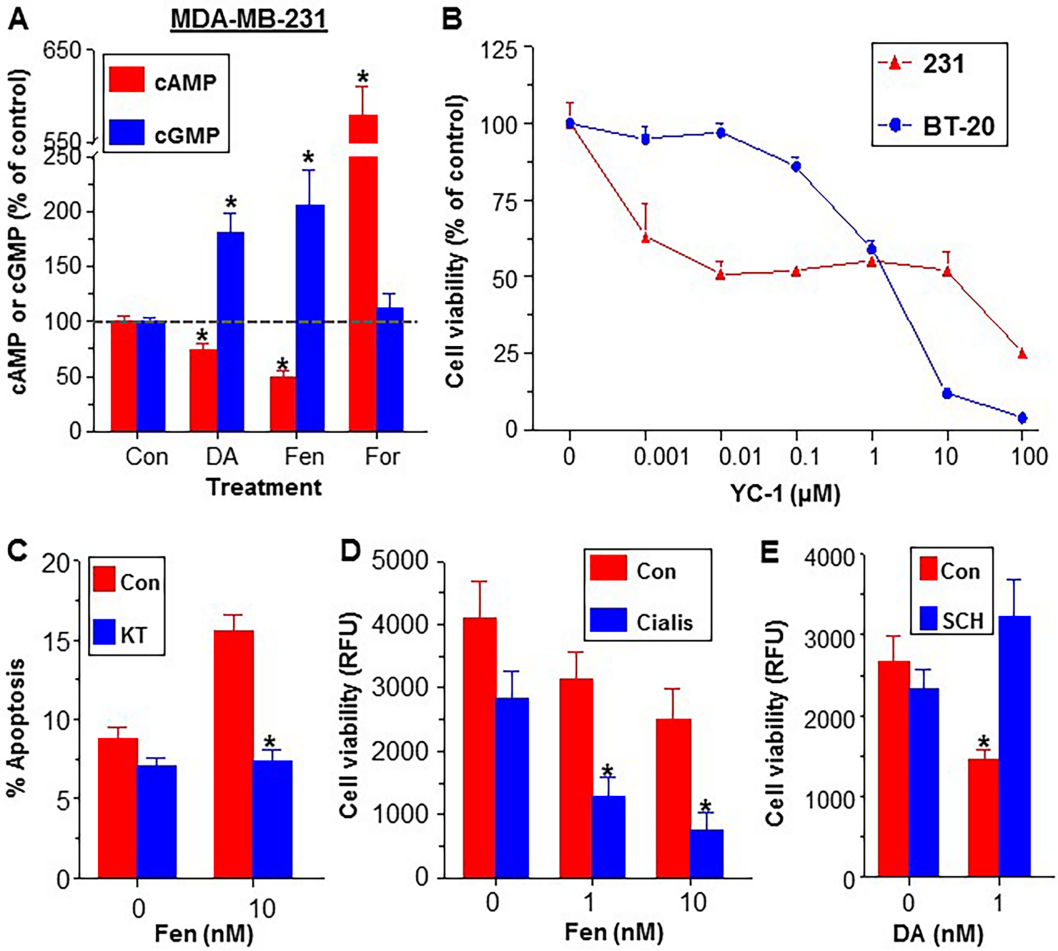

FIG. 11: Activation of the cGMP/PKG signaling pathway in breast cancer cells by DA and fenoldopam (Fen). (A) Suppression of cAMP and stimulation of cGMP in MDA-MB-231 cells incubated with 10 nM DA or Fen for 60 min; forskolin (For; 10 μM) served as a positive control for cAMP. (B) YC-1, a sGC activator, suppressed MDA-MB-231 (231) and BT-20 cell viability. (C) KT5823 (KT), a PKG inhibitor, abrogated Fen-induced apoptosis. MDA-MB-231 cells were pre-incubated with 5 μM KT for 30 min, followed by incubation with 10 nM Fen for 48 hrs; apoptosis was determined by TUNEL. (D) Cialis (1 μM), a PDE5 inhibitor, augmented the ability of FEN to suppress the viability of SUM159 cells. (E) SCH39166 (SCH), a D1R antagonist, abrogated DA-induced inhibition of cell viability. *$p < 0.05$. Adapted from Borcherding et al.[33]

injections of human anti-D1R antibody conjugated to Alexa-Fluor 647 resulted in an intense fluorescence of the primary tumors in the mammary fat pads and axillary metastases in mice bearing MDA-MB-231-derived xenografts.

An involvement of DAR in BC was reported in a recent study[55] that have examined the effects of several phenothiazines on triple negative BCC. Phenothiazines are antipsychotics that bind to DAR (D1R and D2R), but also to muscarinic, histaminergic and serotonergic receptors. The phenothiazines reduced cell proliferation and invasion, and increased cell death *in vitro*, while their administration to mice bearing MDA-MB-231 xenografts reduced tumor growth and metastatic burden.

D. Therapeutic Applications

Identification of BC patients as potential candidates for DAR-targeted therapy could be accomplished

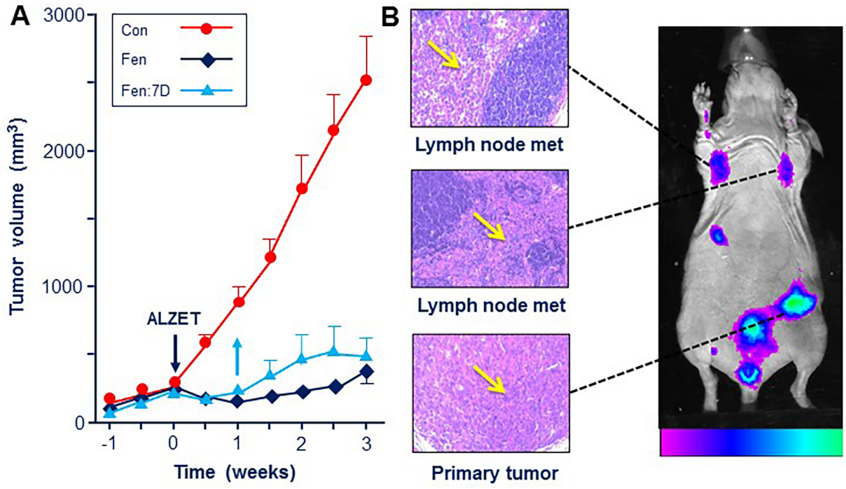

FIG. 12: Suppression of breast cancer xenograft growth by fenoldopam (Fen) and tumor D1R imaging. (A) Treatment with Fen, delivered by Alzet osmotic mini-pumps, markedly reduced the growth of SUM159-derived xenografts in athymic nude mice. One group (Fen) had the pumps for 3 weeks, while another group (Fen 7D), had the pumps removed after 7 days. (B) Fluorescence imaging of D1R-expressing xenografts. Mice with MDA-MB-231-derived tumors were i.v. injected with human anti-D1R antibody conjugated to Alexa-Fluor 647. *In vivo* fluorescence imaging after 24 h shows intense fluorescence in both primary tumor and metastases. Insets show histological preparations of primary tumor and axillary lymph node metastases. Adapted from Borcherding et al.[33]

using two approaches: (1) analysis of DAR immunostaining in tumor biopsies, as is routinely done for ER and HER2; and (2) positron emission tomography (PET) imaging, which is effectively used to diagnose neuropsychiatric disorders such schizophrenia, Parkinson's disease, and drug dependence.[56] PET uses positron-emitting radioisotopes to image tumors with high sensitivity and excellent resolution. Among the various PET ligands, ^{124}I TISCH is clinically used for selective D1R imaging. PET is based on a functional readout of tumor properties such as high metabolism, and expression of unique receptors, with the ultimate goal of identifying effective therapeutic targets. The advantages of PET imaging include (1) the ability to assess receptor expression for the entire tumor burden, thus avoiding some sampling errors that occur with heterogeneous receptor expression in primary tumors; (2) a non-invasive assessment of distal metastases that are not accessible to sampling by biopsy; and (3) a serial monitoring of drug effects on designated targets.

Among dozens of agonists and antagonists with high selectivity for DAR subtypes, only a few, exemplified by fenoldopam, do not cross the BBB and target only peripheral D1R. Fenoldopam is a small molecule with a mass of 305 g/mol. Small molecules have proven highly valuable for treating many diseases, and most oral medicines marketed today belong to this class. Although fenoldopam is currently delivered to patients with renal hypertension by infusion, pharmaceutical companies can likely develop slow release formulation with prolonged activity and oral deliverability. In our studies we also identified two FDA-approved drugs that bypass the D1R as potential novel therapeutics in BC. Riociguat is available in a tablet form to treat chronic pulmonary hypertension, while Cialis is used as an oral medication to treat erectile dysfunction. In addition to its ability to suppress tumor growth, Cialis boosts the capacity of the immune system to eliminate cancer cells.[57]

VIII. SUMMARY AND PERSPECTIVES

Each year, over 280,000 women in the United States are diagnosed with invasive BC, and 43,000 will die from the disease. Although therapies against ER and

HER2 have improved response rate and survival, patients with advanced disease, who are resistant to anti-hormonal therapy and/or to chemotherapy, have limited treatment options for reducing morbidity and mortality. These shortcomings provide major incentives to develop new, effective, and personalized therapies. We propose that the D1R, which is overexpressed in ~ 30% of BC, is a prospective prognostic factor, and a novel therapeutic target. D1R in BC signals via the cGMP/PKG pathway, and its activation results in apoptosis, inhibition of cell invasion, increased sensitivity to doxorubicin, and suppression of xenograft growth.

Our working model (Fig. 13) stipulates that D1R activation increases inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO). NO activates sGC and increases cGMP production, followed by PKG activation and apoptosis. Agents that affect cGMP levels include (1) fenoldopam, a high affinity peripheral D1R agonist; (2) YC-1 and riociguat, which activate sGC; and (3) Cialis, which inhibits PDE5 and prevents cGMP hydrolysis. Fenoldopam, Cialis, and riociguat are FDA-approved to treat renal hypertension, erectile dysfunction, and pulmonary hypertension, respectively, and could be repurposed to treat BC patients.

Important Note: Upon exploring the Oncomine database (www.oncomine.org), we found multiple datasets, including The Cancer Genome Atlas (TCGA), reporting on *DRD1* gene expression in normal and malignant tissues. We found highly variable data, some of which agreed with, while others did not agree with, our results on D1R overexpression in advanced BC. Since the *DRD1* gene is unusual in that it has no introns, the use of inappropriate probes in microarrays, or some genomic contamination of the specimens, could explain some of the inconsistent data. In our RT-PCR analysis of *DRD1* expression in BCC and primary carcinomas, we were especially careful to verify lack of genomic contamination.

We also found highly variable data on D1R protein staining in histological sections of tumors in the Human Protein Atlas collection (www.proteinatlas.org). As was previously reported,[58] and confirmed by us, many of the commercially available Abs against D1R lack specificity. Therefore, before we embarked on analysis of D1R staining in tissue

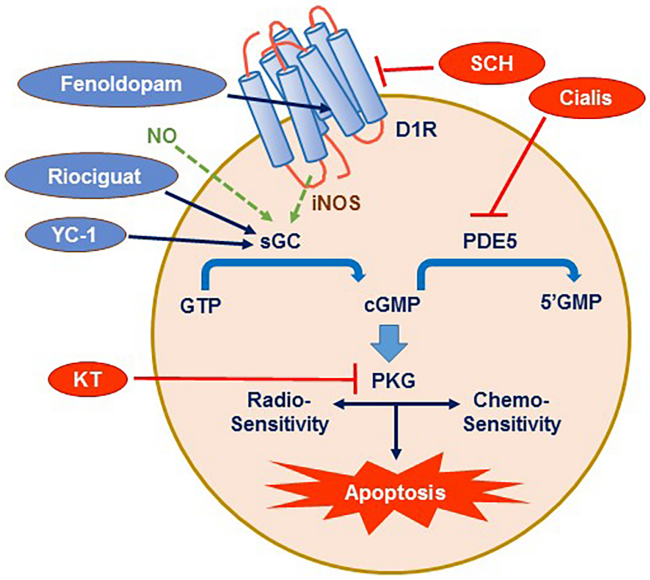

FIG. 13: Proposed model of the interactions of D1R with the cGMP/PKG system in breast cancer. The association of D1R with iNOS (inducible nitric oxide synthase) is assumed but not proven. NO: nitric oxide; SCH: SCH39166, a D1R inhibitor; KT: KT5823, a PKG inhibitor; sGC: soluble guanylate cyclase. See text for other explanations.

microarrays, we tested and rejected 4–5 Abs. As detailed in our *Oncogene* paper,[33] we then rigorously validated a rabbit mAb, using adsorption with the immunizing peptide, elimination of the protein band following *DRD1* knockdown, and abolishment of the apoptotic response to fenoldopam in D1R-deficient cells. We recently generated our own mouse mAb, directed against the third extracellular loop of the D1R protein. Initial characterization showed excellent selectivity and high affinity of this mAb. Hopefully, this mAb can be used for developing a diagnostic kit for analyzing tumor biopsies from BC patients for D1R expression.

IX. A TRIBUTE TO PETER STAMBROOK

Peter was the chair of the Department of Cancer and Cell Biology at the University of Cincinnati from 1996 to 2008. Throughout this time he was a colleague, a friend, and a great supporter of our laboratory. Under his leadership and continuous encouragement, we made the transition from neuroendocrinologists to cancer biologists. All of us who knew Peter marveled at his superb knowledge and true enthusiasm for science. He is well remembered for his kindness and humanity. We will forever be grateful for the opportunity to have known him, and we miss him terribly.

REFERENCES

1. Ben-Jonathan N. Dopamine: Endocrine and oncogenic functions. Boca Raton, FL: CRC Press; 2020.
2. Rubi B, Maechler P. Minireview: New roles for peripheral dopamine on metabolic control and tumor growth: Let's seek the balance. Endocrinology. 2010;151(12): 5570–81.
3. Borcherding DC, Hugo ER, Idelman G, Richtand NW, Loftus J, Ben-Jonathan N. Dopamine receptors in human adipocytes: Expression and functions. PLoS One. 2011;6(9):e25537.
4. Marengo FD, Cardenas AM. How does the stimulus define exocytosis in adrenal chromaffin cells? Pflugers Arch. 2018;470(1):155–67.
5. Goldstein DS, Swoboda KJ, Miles JM, Coppack SW, Aneman A, Holmes C, Lamensdorf I, Eisenhofer G. Sources and physiological significance of plasma dopamine sulfate. J Clin Endocrinol Metab. 1999;84(7):2523–31.
6. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of biogenic amines in food—existing and emerging approaches. J Food Sci. 2010;75(7):R139–R50.
7. Kawamura M, Schwartz JP, Nomura T, Kopin IJ, Goldstein DS, Huynh TT, Hooper DR, Harvey-White J, Eisenhofer G. Differential effects of chemical sympathectomy on expression and activity of tyrosine hydroxylase and levels of catecholamines and DOPA in peripheral tissues of rats. Neurochem Res. 1999;24(1):25–32.
8. Strott CA. Sulfonation and molecular action. Endocr Rev. 2002;23(5):703–32.
9. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: From structure to function. Physiol Rev. 1998;78(1):189–225.
10. Park JY, Lee SY, Kim HR, Seo MD, Chung KY. Structural mechanism of GPCR-arrestin interaction: Recent breakthroughs. Arch Pharm Res. 2016;39(3):293–301.
11. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev. 2011;63(1):182–217.
12. Missale C, Fiorentini C, Collo G, Spano P. The neurobiology of dopamine receptors: Evolution from the dual concept to heterodimer complexes. J Recept Signal Transduct Res. 2010;30(5):347–54.
13. Shaywitz AJ, Greenberg ME. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem. 1999;68:821–61.
14. Belkhiri A, Zhu S, El-Rifai W. DARPP-32: From neurotransmission to cancer. Oncotarget. 2016;7(14):17631–40.
15. Avanes A, Lenz G, Momand J. Darpp-32 and t-Darpp protein products of PPP1R1B: Old dogs with new tricks. Biochem Pharmacol. 2019;160:71–9.
16. Ben-Jonathan N, Borcherding DC, Fox SR, Hugo ER. Activation of the cGMP/protein kinase G system in breast cancer by dopamine receptor-1. Cancer Drug Resistance. 2019;2:933–47.
17. Sharma RK, Duda T. Membrane guanylate cyclase, a multimodal transduction machine: History, present, and future directions. Front Mol Neurosci. 2014;7:article 56.
18. Wang X, Robinson PJ. Cyclic GMP-dependent protein kinase and cellular signaling in the nervous system. J Neurochem. 1997;68(2):443–56.
19. Azevedo MF, Faucz FR, Bimpaki E, Horvath A, Levy I, de Alexandre RB, Ahmad F, Manganiello V, Stratakis CA. Clinical and molecular genetics of the phosphodiesterases (PDEs). Endocr Rev. 2014;35(2):195–233.
20. Supuran CT, Mastrolorenzo A, Barbaro G, Scozzafava A. Phosphodiesterase 5 inhibitors—drug design and differentiation based on selectivity, pharmacokinetic and efficacy profiles. Curr Pharm Des. 2006;12(27):3459–65.
21. Zhu B, Strada SJ. The novel functions of cGMP-specific phosphodiesterase 5 and its inhibitors in carcinoma cells and pulmonary/cardiovascular vessels. Curr Top Med Chem. 2007;7(4):437–54.
22. Scatena R, Martorana GE, Bottoni P, Botta G, Pastore P, Giardina B. An update on pharmacological approaches to

neurodegenerative diseases. Expert Opin Investig Drugs. 2007;16(1):59–72.
23. Strange PG. Antipsychotic drug action: Antagonism, inverse agonism or partial agonism. Trends Pharmacol Sci. 2008;29(6):314–21.
24. Baumeister AA, Francis JL. Historical development of the dopamine hypothesis of schizophrenia. J Hist Neurosci. 2002;11(3):265–77.
25. Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(7): 1081–90.
26. Seeman P. Atypical antipsychotics: Mechanism of action. Can J Psychiatry. 2002;47(1):27–38.
27. Glickstein SB, Schmauss C. Dopamine receptor functions: Lessons from knockout mice [corrected]. Pharmacol Ther. 2001;91(1):63–83.
28. Pardridge WM. The blood-brain barrier: Bottleneck in brain drug development. NeuroRx. 2005;2(1):3–14.
29. Reddymasu SC, Soykan I, McCallum RW. Domperidone: Review of pharmacology and clinical applications in gastroenterology. Am J Gastroenterol. 2007;102(9): 2036–45.
30. Parkman HP, Mishra A, Jacobs M, Pathikonda M, Sachdeva P, Gaughan J, Krynetskiy E. Clinical response and side effects of metoclopramide: Associations with clinical, demographic, and pharmacogenetic parameters. J Clin Gastroenterol. 2012;46(6):494–503.
31. Mathur VS, Swan SK, Lambrecht LJ, Anjum S, Fellmann J, McGuire D, Epstein M, Luther RR. The effects of fenoldopam, a selective dopamine receptor agonist, on systemic and renal hemodynamics in normotensive subjects. Crit Care Med. 1999;27(9):1832–7.
32. Murphy MB, Murray C, Shorten GD. Fenoldopam: A selective peripheral dopamine-receptor agonist for the treatment of severe hypertension. N Engl J Med. 2001;345(21):1548–57.
33. Borcherding DC, Tong W, Hugo ER, Barnard DF, Fox S, LaSance K, Shaughnessy E, Ben-Jonathan N. Expression and therapeutic targeting of dopamine receptor-1 (D1R) in breast cancer. Oncogene. 2016;35(24):3103–13.
34. Streff H, Profato J, Ye Y, Nebgen D, Peterson SK, Singletary C, Arun BK, Litton JK. Cancer incidence in first- and second-degree relatives of BRCA1 and BRCA2 mutation carriers. Oncologist. 2016;21(7):869–74.
35. Schnitt SJ. Classification and prognosis of invasive breast cancer: From morphology to molecular taxonomy. Mod Pathol. 2010;23(Suppl 2):S60–S4.
36. Coley HM. Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. Cancer Treat Rev. 2008;34(4):378–90.
37. Garcia-Aranda M, Redondo M. Immunotherapy: A challenge of breast cancer treatment. Cancers. 2019;11(12); 1822–35.
38. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015;21(1):24–33.
39. Huang B, Warner M, Gustafsson JA. Estrogen receptors in breast carcinogenesis and endocrine therapy. Mol Cell Endocrinol. 2015;418 Pt 3:240–4.
40. Fan W, Chang J, Fu P. Endocrine therapy resistance in breast cancer: Current status, possible mechanisms and overcoming strategies. Future Med Chem. 2015;7(12):1511–9.
41. Jacobson EM, Hugo ER, Borcherding DC, Ben-Jonathan N. Prolactin in breast and prostate cancer: Molecular and genetic perspectives. Discov Med. 2011;11(59): 315–24.
42. Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. Endocr Rev. 2001;22(6):724–63.
43. Sachlos E, Risueno RM, Laronde S, Shapovalova Z, Lee JH, Russell J, Malig M, McNicol JD, Fiebig-Comyn A, Graham M, Levadoux-Martin M, Lee JB, Giacomelli AO, Hassell JA, Fischer-Russell D, Trus MR, Foley R, Leber B, Xenocostas A, Brown ED, Collins TJ, Bhatia M. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell. 2012;149(6):1284–97.
44. Yong L, Yao Y, Chen GS, Yan XX, Guo YC, Han MY, Xue JS, Jian WZ, Zhou TY. QAP14 suppresses breast cancer stemness and metastasis via activation of dopamine D1 receptor. Acta Pharmacol Sin. 2022; doi: 10.1038/s41401-021-00701-9. Online ahead of print.
45. Gholipour N, Ohradanova-Repic A, Ahangari G. A novel report of MiR-4301 induces cell apoptosis by negatively regulating DRD2 expression in human breast cancer cells. J Cell Biochem. 2018;119(8):6408–17.
46. Bakhtou H, Olfatbakhsh A, Deezagi A, Ahangari G. The expression of dopamine receptors gene and their potential role in targeting breast cancer cells with selective agonist and antagonist drugs. Could it be the novel insight to therapy? Curr Drug Discov Technol. 2019;16(2):184–97.
47. Idirisinghe PK, Thike AA, Cheok PY, Tse GM, Lui PC, Fook-Chong S, Wong NS, Tan PH. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer. Pathologic correlations and clinical significance. Am J Clin Pathol. 2010;133(3):416–29.
48. Pornour M, Ahangari G, Hejazi SH, Ahmadkhaniha HR, Akbari ME. Dopamine receptor gene (DRD1-DRD5) expression changes as stress factors associated with breast cancer. Asian Pac J Cancer Prev. 2014;15(23): 10339–43.
49. Belkhiri A, Dar AA, Peng DF, Razvi MH, Rinehart C, Arteaga CL, El-Rifai W. Expression of t-DARPP mediates trastuzumab resistance in breast cancer cells. Clin Cancer Res. 2008;14(14):4564–71.
50. Christenson JL, Denny EC, Kane SE. t-Darpp overexpression in HER2-positive breast cancer confers a survival advantage in lapatinib. Oncotarget. 2015;6(32):33134–45.

51. Denny EC, Kane SE. t-Darpp promotes enhanced EGFR activation and new drug synergies in Her2-positive breast cancer cells. PLoS One. 2015;10(6):e0132267.
52. Ji J, Sundquist K, Ning Y, Kendler KS, Sundquist J, Chen X. Incidence of cancer in patients with schizophrenia and their first-degree relatives: A population-based study in Sweden. Schizophr Bull. 2013;39(3):527–36.
53. Fallahian F, Karami-Tehrani F, Salami S, Aghaei M. Cyclic GMP induced apoptosis via protein kinase G in oestrogen receptor-positive and -negative breast cancer cell lines. FEBS J. 2011;278(18):3360–9.
54. Sarkar C, Chakroborty D, Mitra RB, Banerjee S, Dasgupta PS, Basu S. Dopamine in vivo inhibits VEGF-induced phosphorylation of VEGFR-2, MAPK, and focal adhesion kinase in endothelial cells. Am J Physiol Heart Circ Physiol. 2004;287(4):H1554–H60.
55. Goyette MA, Cusseddu R, Elkholi I, Abu-Thuraia A, El-Hachem N, Haibe-Kains B, Gratton JP, Cote JF. AXL knockdown gene signature reveals a drug repurposing opportunity for a class of antipsychotics to reduce growth and metastasis of triple-negative breast cancer. Oncotarget. 2019;10 (21):2055–67.
56. Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature. 1997;385(6617):634–6.
57. Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W, Dolcetti L, Bronte V, Borrello I. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. J Exp Med. 2006;203(12):2691–702.
58. Bodei S, Arrighi N, Spano P, Sigala S. Should we be cautious on the use of commercially available antibodies to dopamine receptors? Naunyn Schmiedebergs Arch Pharmacol. 2009;379(4):413–5.