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Minireviews

Cancer and the Dopamine D_2 Receptor: A Pharmacological Perspective

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ABSTRACT

The dopamine D_2 receptor (D_2R) family is upregulated in many cancers and tied to stemness. Reduced cancer risk has been correlated with disorders such as schizophrenia and Parkinson's disease, in which dopaminergic drugs are used. D_2R antagonists are reported to have anticancer efficacy in cell culture and animal models where they have reduced tumor growth, induced autophagy, affected lipid metabolism, and caused apoptosis, among other effects. This has led to several hypotheses, the most prevalent being that D_2R ligands may be a novel approach to cancer chemotherapy. This hypothesis is appealing because of the large number of approved and experimental drugs of this class that could be repurposed. We review the current state of the literature and the evidence for and against this hypothesis. When the existing literature is evaluated from a pharmacological context, one of the striking findings is that the concentrations needed for cytotoxic effects of D_2R antagonists are orders of magnitude higher than their affinity for this receptor. Although additional definitive studies will provide further clarity, our hypothesis is that targeting D_2-like dopamine receptors may only yield useful ligands for cancer chemotherapy in rare cases.

The Concatenation of Cancer and Neuropharmacology

The serendipitous discovery of chlorpromazine (Delay et al., 1952; Delay and Deniker, 1955) over 60 years ago may be considered a landmark in several ways. Besides offering the first effective treatment of some of the symptoms of schizophrenia, it opened new doors to an understanding of the chemoarchitecture of the brain, especially the role of dopamine (Carlsson et al., 1958; Carlsson and Lindqvist, 1963). This led to millions of people being treated with drugs that targeted dopamine receptors. In psychiatry, this complicated a decades-long debate about whether schizophrenia itself affected cancer risk. For a review, see Gulbinat et al. (1992), who noted that pharmacological mechanisms were of particular interest, especially because some phenothiazine-based drugs had antitumor activity in murine leukemia and melanoma, and high concentrations of the antipsychotics or their metabolites were found in the lung (Driscoll et al., 1978). These latter findings might explain a lower occurrence of malignancies sometimes reported in schizophrenics. Conversely, because classic antipsychotics markedly increased serum prolactin resulting from antagonism of inhibitory dopamine receptors on anterior pituitary lactotrophs, this also might explain an increased risk of breast cancer in females

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ABBREVIATIONS: Akt, protein kinase B; AML, acute myeloid leukemia; AraC, Cytosine arabinoside; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid acetoxymethyl ester; CHO, Chinese hamster ovary; CSCs, cancer stem cells; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; FOX, Forkhead box; GFP, green fluorescent protein; GBM, glioblastoma; 10 GPCR, G protein-coupled receptor; Go6976, 5,6,7,13-Tetrahydro-13-methyl-5-oxo-12H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-12-propanenitrile; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; L-741,626, 3-[4-(4-chlorophenyl)-4-hydroxypiperidinyl]methylindole; LXR, liver X receptors; MAPK, mitogen-activated protein kinase; NGB2904, *N*-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyl]-9*H*-fluorene-2-carboxamide; ONC201, 7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(4H)-one; PD, Parkinson disease; PG01037, *N*-[(2*E*)-4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-2-buten-1-yl]-4-(2-pyridyl)-benzamide; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PNU 96415E, 1-[2-(3,4-dihydro-1*H*-2-benzopyran-1-yl)ethyl]-4-(4-fluorophenyl)piperazine; PPAR, Peroxisome proliferator-activated receptor; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; shRNA, short-hairpin RNA; siRNA, short-interfering RNA; ROS, reactive oxygen species; SB-277011A, N-{trans-4-[2-(6-cyano-3,4-dihydroisoquinolin-2(1H)-yl)ethyl]cyclohexyl}quinoline-4-carboxamide; SCZ, Schizophrenia; SKF-83,959, 6-Chloro-2,3,4,5-tetrahydro-3-methyl-1-(3-methylphenyl)-1H-3-benzazepine-7,8-diol; SREBP, sterol regulatory element-binding proteins; STAT, signal transducer and activator of transcription; TRAIL, TNF-related apoptosis-inducing ligand; U99194, 2,3-dihydro-5,6-dimethoxy-*N*, *N*-dipropyl-1*H*-inden-2-amine; VEGFR, vascular EGFR.

(Gulbinat et al., 1992). These early observations led to the hypotheses, first suggested in 1972, that dopamine agonists (then all of the D2 type) might be a potential therapeutic approach in cancer (Csatary, 1972), as will be discussed later.

Dopamine Receptors

Dopamine receptors are members of the heptahelical G protein-coupled receptor (GPCR) superfamily and are divided pharmacologically into two subfamilies (Fig. 1): "D_1-like" and "D_2-like" (Garau et al., 1978; Kebabian and Calne, 1979). The molecular biology and pharmacology of these receptors have been the subject of numerous reviews and books (Neve and Neve, 1997; Mailman and Huang, 2007). Dopamine receptors are encoded by five genes, with *DRD1* and *DRD5* encoding the two D_1-like receptors (D_1 and D_5), and *DRD2*, *DRD3*, and *DRD4* encoding four expressed mammalian proteins (D_{2long}, D_{2short}, D_3, and D_4). D_{2long} and D_{2short} are splice variants from *DRD2* and together are the most highly expressed of the D_2-like receptors (Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989b; Chio et al., 1990). As noted earlier, the first drugs that were shown to bind to dopamine receptors (e.g., chlorpromazine) were discovered serendipitously because of effects in controlling positive symptoms of schizophrenia. The target of early antipsychotic drugs was soon identified, then validated, via radioreceptor studies and receptor cloning (Burt et al., 1976; Seeman et al., 1976; Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989a, 1990). When using drugs as research tools, it is imperative to understand the relative effects of a molecule on both primary and secondary targets; antipsychotics in particular have many off-target actions. In addition, although they may have selectivity for one subfamily of dopamine receptor, there is often much less selectivity for an individual member (e.g., D_2 vs. D_3 vs. D_4). Thus, when we discuss clinical findings, reference to "D_2" will be a reference to D_2-like affinity unless otherwise specified.

There is a rich literature on both agonist and antagonist effects on dopamine receptors, but it has largely been focused on central nervous system modulation of dopamine function in the context of schizophrenia and other brain disorders (Neve and Neve, 1997). On the periphery, dopamine is known to play an important role in cardiovascular control and kidney function. The notion that dopamine receptor ligands might affect the biology of neoplastic cells independent of their actions on neurotransmission is provocative, and offers both a novel mechanism and the ability to both purpose and repurpose the huge libraries of dopaminergic ligands and drugs that have resulted from neuropharmacological drug discovery and development (Schalop and Allen, 2016). Thus, an examination of this arena is timely.

Clinical Studies of Dopaminergic Drugs and Cancer

Correlative Studies and Case Reports Support a Role for the D_2 Receptor in Cancer Development and Treatment Response. To date, all antipsychotic drugs engage D_2 receptors, usually as antagonists (Creese et al., 1976; Mailman, 2007; Boyd and Mailman, 2012), whereas therapy for Parkinson disease (PD) relies primarily on activation of dopamine receptors indirectly via levodopa, or directly by direct agonists (Mailman and Huang, 2007). The accepted targets of current dopamine agonists in PD have been the D_2 and D_3 receptors. Although some findings suggest a greater role for D_1 receptors (Taylor et al., 1991; Mailman et al., 2001), the clinical data of relevance to this topic deals with D_2R-targeted therapeutics.

Investigations into the relationship between D_2R antagonists and cancer began almost as soon as these drugs were approved for psychiatric indications (Table 1), starting with isolated case reports of increased treatment response from cancer patients treated concurrently with antipsychotics (Osterman, 1961; Csatary, 1972; Eicke, 1973; Hercbergs, 1988). Correlative studies of cancer risk in the context of other diseases strengthened this anecdotal association (Fig. 2), (Fig. 3), Table 2). By the 1980s, population-based correlative studies to determine cancer risk within groups of patients with schizophrenia and PD were underway. Many studies showed clear, significant differences in cancer development, yet methodologies were quite variable, and cohorts often small. Some studies were prospective and followed matched cohorts,

Dopamine receptors

D1-like (- Gαs coupled)		D2-like (- Gαi/o coupled)		
D1	**D5**	**D2**	**D3**	**D4**
Substantia nigra Nucleus accumbens Olfactory bulb Lower levels: Cerebellum Hippocampus Thalamus Kidney	Substantia nigra Hypothalamus Kidney Heart Sympathetic ganglia	Substantia nigra Nucleus accumbens Ventral tegemental area Lower levels: Heart Blood vessels Adrenal glands Sympathetic ganglia	Olfactory bulb Nucleus accumbens	Heart Blood vessels Substantia nigra Hippocampus Amygdala Gastrointestinal tract

Fig. 1. Dopamine receptors are G protein-coupled receptors, which are divided into the D_1- and D_2-like families. Some tissues of interest where these receptors are expressed are included here.

TABLE 1
Timeline of D_2 receptor pharmacology and early cancer findings

Year	Event	Source
1950	Chlorpromazine synthesized	
1952	Chlorpromazine identified as antipsychotic	Delay et al. (1952), Sigwald and Bouttier (1953)
1959	Reactive oxygen species are associated with antipsychotics at millimolar concentrations.	Dawkins et al. (1959)
1961–1988	First published case reports of increased sensitivity to chemotherapy with concurrent antipsychotic treatment.	Osterman (1961)
1976–1979	Dopamine receptor families proposed (D_1-like, D_2-like)	Garau et al. (1978), Kebabian and Calne (1979)
1986	Phenothiazines can reverse doxorubicin resistance in KB cells.	Akiyama et al. (1986)
1988–1989	D_2-like receptors cloned (human and rodent).	Bunzow et al. (1988)
1990	Pimozide and thioridazine reduce breast cancer cell proliferation	Strobl et al. (1990)
1991	Radiation sensitization of bone marrow under concurrent chlorpromazine treatment.	Jagetia and Ganapathi (1991)
1992	Bromocriptine (D_2 agonist) increases cancer growth and proliferation (rat gastric carcinogenesis model).	Iishi et al. (1992)
1993	*DRD2* gene linked to breast cancer via linkage study in a single family lacking *BRCA1* deficiency.	Cortessis et al. (1993)
1994	D_2R are present and inducible by retinoic acid in SH-SY-5Y neuroblastoma cells.	Farooqui et al. (1994)

whereas others mined national healthcare databases. These differences complicate arriving at a unitary hypothesis.

Of particular note was a study of more than 100,000 age- and gender-matched, primarily Han Chinese schizophrenia patients in which both male and female subjects showed a strong inverse correlation for age and development of cancers (Wu et al., 2013). One possible explanation for this trend is that older patient populations had undergone long-term treatment with neuroleptic agents that might have attenuated the increased risk inherent in schizophrenics. This study was limited, however, by the lack of ethnic diversity, as well as the lack of stratification for other risk factors, such as smoking status.

The D_2 Receptor Is Expressed in a Number of Cancer Cell Lines and in Patient Samples. D_2 receptor expression has been reported at both the mRNA and protein levels in a variety of cancers. Increased immunohistochemical staining has been reported in cervical, esophageal, and lung cancers, often correlating with tumor grade or survival (Li et al., 2006; Hoeppner et al., 2015; Kanakis et al., 2015; Mao et al., 2015; Cherubini et al., 2016). In acute myeloid leukemia (AML), D_2R protein is also highly expressed. *DRD2* mRNA levels are elevated in breast cancer (Pornour et al., 2014), ovarian cancer (Moreno-Smith et al., 2011), glioma (Li et al., 2014), and neuroblastoma (Deslauriers et al., 2011). Peripheral blood mononuclear cells of breast cancer patients express *DRD1*, *DRD2*, *DRD3*, and *DRD4* mRNA (Pornour et al., 2014). Because the D_3R and D_4R have significant homology to the D_2R and often recognize the same drugs, these D_2-like receptors may also be relevant.

It is a reasonable hypothesis that tumors derived from cells in which dopamine plays a clear D_2-mediated inhibitory role (e.g., from the pituitary, etc.) would be inhibited by D_2 agonists. Indeed, the early suggestions to this effect (Csatary, 1972; Jacobs and Franks, 1975) have led to the use of dopamine agonists as one mechanism for controlling such tumors, in which a clear role of dopamine receptors can be demonstrated (Hoeppner et al., 2015), sometimes involving effects on angiogenesis (Chauvet et al., 2017). In clinical, and many of laboratory in vivo, studies of such uses of dopamine agonists, the doses used, after allometric adjustment, are consistent with mediation via the D_2 receptor rather than off-target effects. Yet whereas targeting certain types of tumors with dopamine agonists has a sound physiologic rationale, many

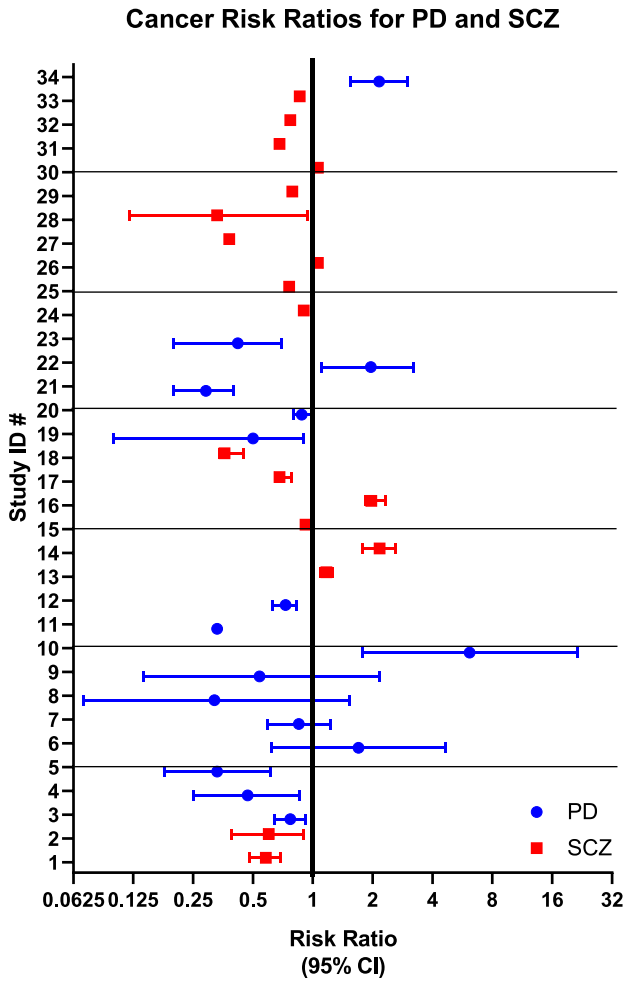

Fig. 2. Forest plot of risk ratios from Table 2, by ID number. Bars represent 95% confidence intervals. Studies of PD patients are shown in blue, and studies of SCZ patients are in red.

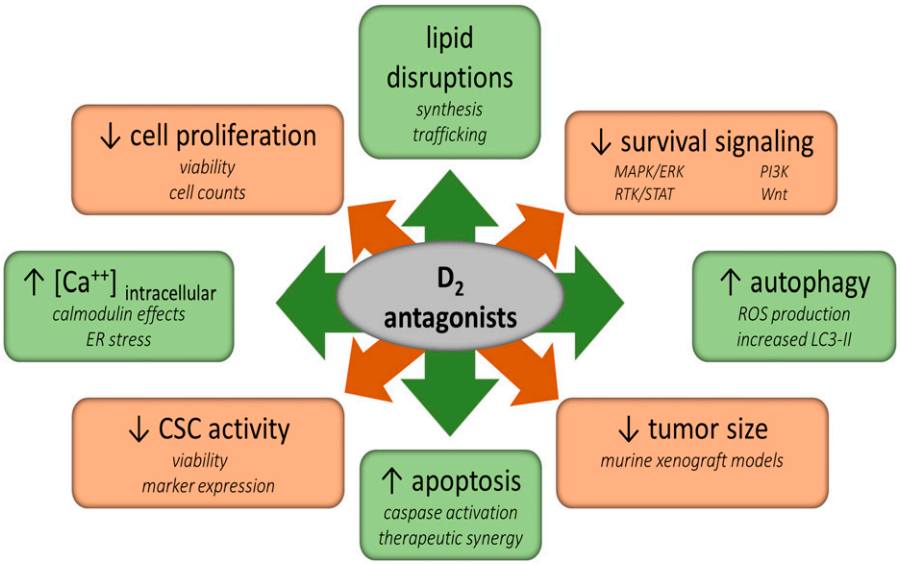

Fig. 3. Treatment with D_2 antagonists affects many vital metabolic processes within cancer cells and tumors. Cancer stem cell-like activities, survival signaling, and proliferation are reduced by treatment. However, intracellular calcium levels, autophagy, and apoptosis are increased. Additionally, lipid synthesis and trafficking are disrupted. The direct mechanisms by which these alterations occur is not currently known, but these compounds may ultimately lead to cell death through these or other pathways.

of the studies ascribing roles for dopamine receptors have important limitations: the use of small numbers of patient samples, lack of blinding, and use of antibodies with poor specificity (Stojanovic et al., 2017). Few studies have ascertained both protein and mRNA levels of the D_2R, and no histochemical studies have published replicate data with other probes to verify selectivity. Importantly, reported mRNA levels have typically been quite low, so large fold-changes in mRNA presence may have little functional impact. Thus, although many studies have reported potential anticancer efficacy of dopamine receptor ligands, a large number have failed to show definitive presence of D_2R protein or message, especially when the drugs being studied were antagonists. We shall explore these important issues below.

Cancer and the "Non-Neuropharmacology" of Dopamine Receptor Ligands

Some of the earliest indications of anticancer activity for D_2R ligands were from Driscoll et al. (1978) and Akiyama et al. (1986). Micromolar concentrations of phenothiazine antipsychotics reversed KB-cell resistance to doxorubicin, vinblastine, dactinomycin, and daunorubicin in a noncalmodulin-dependent manner (Akiyama et al., 1986). In contrast, another study concluded that a reduced proliferative effect of the D_2-like antagonists thioridazine and pimozide in Michigan Cancer Foundation (MCF)-7 cells was attributable to calmodulin antagonism (Strobl et al., 1990). Yet, Iishi et al. (1992) soon reported that the D_2-like agonist bromocriptine promoted gastric carcinogenesis in a rat model, shortly followed by the suggestion of genetic linkage between the *DRD2* gene and BRCA1-sufficient breast cancer (Cortessis et al., 1993). Although these early studies hinted at a potential role for D_2R antagonism in cancer development and treatment, there are some issues that should be considered in interpreting these data. In particular, the effects of the four antipsychotic drugs noted above required concentrations two or more orders-of-magnitude higher than their K_D (Table 3).

Large-Scale Screens Have Identified D_2R as a Potential Target for Anticancer Therapies. Since 2003, several screening studies identified D_2R antagonists as potential therapeutics for cancer treatment on the basis of their biologic activity and/or presence in cancer cells. Like calmodulin inhibitors, phenothiazines selectively increased Forkhead box (FOX)O transcription factor nuclear localization in 786-O renal cell adenocarcinoma cells (Kau et al., 2003), yet FOXO localization remained unchanged when treated with D_2R antagonists of different chemotypes (i.e., clozapine and haloperidol) to control for off-target effects. Although this suggests that the D_2R is not involved, it contrasts with previous reports noting that D_2R agonist treatment increases phospho-Akt levels in neurons, an effect that would be expected to exclude FOXO from the nucleus (Brami-Cherrier et al., 2002; Kihara et al., 2002). Nuclear localization and transcriptional activity of FOXO3 in the human breast cancer BT549 cell line, however, was increased by 5 μM-concentrations of the calcium channel blocker bepridil or the antipsychotic trifluoperazine (Park et al., 2016).

An in silico screening approach suggested thioridazine may inhibit the Akt/ phosphoinositide 3-kinase (PI3K) pathway as well (Rho et al., 2011). Experimentally, thioridazine (20 μM) decreased PI3K pathway activation, inhibited cell cycle progression at G1, reduced cell viability, and induced apoptosis via caspase-3 cleavage over 24 hours of treatment in a manner that was additive with paclitaxel and cisplatin. This suggested that phenothiazines could impact Akt/PI3K signaling in a cell type–specific manner, but target engagement was not verified and may not involve the D_2R (Rho et al., 2011). More recently, Gutierrez et al. (2014) did dual screening seeking compounds that were toxic toward zebrafish thymocytes that overexpress MYC and synergized with Notch inhibitors in human T-cell acute lymphoblastic leukemia (T-ALL) cells. They identified several phenothiazines (including perphenazine and chlorpromazine) as potential anti-T-ALL treatments that bound protein phosphatase 2A (PP2A) (Gutierrez et al., 2014).

Two large-scale screens identified the D_2R protein itself as a potential target that is upregulated in pancreatic cancer and glioblastoma multiforme. The D_2R and its associated G protein $G_{\alpha i2}$ were highly upregulated in pancreatic ductal adenocarcinoma tissue samples (Jandaghi et al., 2016). In an short-hairpin (sh)RNA screen to identify genes necessary for glioblastoma

TABLE 2
Cancer risk in schizophrenia and Parkinson's disease patients

#	Study	Odds Ratio	95% CI	Type	Cancer Type	Cohort (n)	Age	Sex	Diagnosis	Note
1	Barak et al. (2005)	0.58	0.48–0.69	SIR	All types	3226		M/F	SCZ	
2	Barak et al. (2005)	0.6	0.39–0.90	SIR	Breast	1247		F	SCZ	
3	Becker et al. (2010)	0.77	0.64–0.92	IRR	All types	2993		M/F	PD	
4	Becker et al. (2010)	0.47	0.25–0.86	IRR	Lung	2993		M/F	PD	
5	Becker et al. (2010)	0.33	0.18–0.61	IRR	Lymphoma/ leukemia	2993		M/F	PD	
6	Becker et al. (2010)	1.7	0.62–4.67	IRR	Melanoma	2993		M/F	PD	
7	Driver et al. (2007)	0.85	0.59–1.22	Adjusted RR	All types	487		M/F	PD	Prospective
8	Driver et al. (2007)	0.32	0.07–1.53	Adjusted RR	Lung	487		M/F	PD	Prospective
9	Driver et al. (2007)	0.54	0.14–2.16	Adjusted RR	Colorectal	487		M/F	PD	Prospective
10	Driver et al. (2007)	6.15	1.77–21.37	Adjusted RR	Melanoma	487		M/F	PD	Prospective
11	Jansson and Jankovic (1985)	~0.33		Combined IR	All types	406		M/F	PD	
12	Jespersen et al. (2016)	0.73	0.63–0.83	Adjusted OR	Prostate	45,429		M	PD	Case control
13	Lichtermann et al. (2001)	1.17	1.09–1.25	SIR	All types	26,996		M/F	SCZ	
14		2.17	1.78–2.6	SIR	Lung	26,996		M/F	SCZ	Not controlled for smoking
15	Wu et al. (2013)	0.92	0.9–0.96	SIR	All types	102,202	All	M/F	SCZ	Declines with age
16	Wu et al. (2013)	1.97	1.85–2.33	SIR	All types	102,202	20–29	M/F	SCZ	
17	Wu et al. (2013)	0.68	0.65–0.78	SIR	All types	102,202	60–69	M/F	SCZ	
18	Wu et al. (2013)	0.36	0.34–0.45	SIR	All types	102,202	>70	M/F	SCZ	
19	Hamaue et al. (2000)	<1		SIR	All types	246		M/F	PD	Retrospective, not significant (small sample size)
20	Moller et al. (1995)	0.88	0.8–1.0	Relative risk	All types	7046		M/F	PD	National cohort
21	Moller et al. (1995)	0.29	0.2–0.4	Relative risk	Lung	7046		M/F	PD	
22	Moller et al. (1995)	1.96	1.1–3.2	Relative risk	Melanoma	7046		M/F	PD	
23	Moller et al. (1995)	0.42	0.2–0.7	Relative risk	Bladder	7046		M/F	PD	
24	Munk-Jorgensen and Mortensen (1989)	0.9		IRR	All types	6152	All	M/F	SCZ	
25	Munk-Jorgensen and Mortensen (1989)	0.76		IRR	All types	2956	All	M	SCZ	
26	Munk-Jorgensen and Mortensen (1989)	1.06		IRR	All types	3196	All	F	SCZ	
27	Munk-Jorgensen and Mortensen (1989)	0.38		IRR	Respiratory	6152	All	M/F	SCZ	
28	Eaton et al. (1992)	0.33	0.12–0.94	IRR	Prostate	38	All	M	SCZ	Treated with high dose neuroleptics (e.g., chlorpromazine)
29	Mortensen (1994)	0.79		SIR	All types	9156	All	M/F	SCZ	With SCZ diagnosis
30	Mortensen (1994)	1.06		SIR	All types	5658		M	SCZ	
31	Mortensen (1994)	0.68		SIR	All types	5658		M	SCZ	Before SCZ diagnosis
32	Mortensen (1994)	0.77		SIR	All types	3498		F	SCZ	Before SCZ diagnosis
33	Mortensen (1994)	0.86		SIR	All types	3498		F	SCZ	
34	Wang et al. (2015)	2.16	1.55–2.99	Adjusted OR	All types	6211	All	M/F	PD	Patients prescribed ergot-derived dopamine agonists

SIR, Standardized Risk Ratio; IRR, Incident Rate Ratio; RR, Relative Risk; OR, Odds Ration.

TABLE 3
Ligand affinities of select D_2 antagonists, agonists, and functionally selective ligands (nM)[1]

Compound	D2	Reference Ligand	Source	D1	Reference Ligand	Source	D3	Reference Ligand	Source	D4	Reference Ligand	Soursce	D5	Reference Ligand	Source
Aripiprazole	0.59	^{3}H-NMSP	Lawler et al. (1999)	410	^{3}H-SCH23390	Lawler et al. (1999)	9.7	^{3}H-NMSP	Shapiro et al. (2003)	514	^{3}H-NMSP	Besnard et al. (2012)	1200	^{3}H -SCH23390	Lawler et al. (1999)
Chlorpromazine	2	^{3}H-NMSP	Besnard et al. (2012)	112	^{3}H-SCH23390	Besnard et al. (2012)	1.3	^{3}H-raclopride	Seeman (1995)	24	^{3}H-NMSP	Besnard et al. (2012)	133	^{3}H -SCH23390	Besnard et al. (2012)
Clomipramine	77.6	^{3}H-spiperone	Millan et al. (2001)	219	^{3}H-SCH23390	Toll et al. (1998)	50.1	^{3}H-spiperone	Millan et al. (2001)						
Clozapine	431	^{3}H-NMSP	Besnard et al. (2012)	189	^{3}H-SCH23390	Besnard et al. (2012)	340	^{3}H-nemonapride	Seeman (1995)	39	^{3}H-NMSP	Besnard et al. (2012)	235	^{3}H -SCH23390	Besnard et al. (2012)
Fluphenazine	0.54	^{3}H-NMSP	Besnard et al. (2012)	24	^{3}H-SCH23390	Besnard et al. (2012)	0.3	^{3}H-nemonapride	Seeman (1995)	36	^{3}H-NMSP	Besnard et al. (2012)	12	^{3}H -SCH23390	Besnard et al. (2012)
Haloperidol	2	^{3}H-NMSP	Besnard et al. (2012)	83	^{3}H-SCH23390	Besnard et al. (2012)	23	^{3}H-nemonapride	Seeman (1995)	15	^{3}H-NMSP	Besnard et al. (2012)	147	^{3}H-SCH23390	Besnard et al. (2012)
Olanzapine	72	^{3}H-NMSP	Besnard et al. (2012)	58	^{3}H-SCH23390	Besnard et al. (2012)	40	^{3}H-nemonipride	Seeman (1995)	19	^{3}H-NMSP	Besnard et al. (2012)	90	^{3}H-SCH23390	Besnard et al. (2012)
Penfluridol	5.6	^{3}H-haloperidol	Burt et al. (1976)	1600*	^{3}H-dopamine	Burt et al. (1976)				31**	^{3}H-spiperone	Roth et al. (1995)			
Perphenazine	1.4	^{3}H-NMSP	Burt et al. (1976), Kroeze et al. (2003)	29.9**	^{3}H-SCH23390	Billard et al. (1984)	1.1	^{3}H-nemonapride	Seeman (1995)	32	^{3}H-spiperone	Seeman (1995)			
Pimozide	0.65	^{3}H-NMSP	Kroeze et al. (2003)	$>10^4$	^{3}H-SCH23390	Toll et al. (1998)	11	^{125}I-iodosulpride	Schmidt et al. (1992)	1.8	R-SAT	Burstein et al. (2005)			
Prochlorperazine	4	^{3}H-spiperone	Seeman (1995)				1.8	^{125}I-iodosulpride	Schmidt et al. (1992)	70	^{3}H-spiperone	Seeman (1995)			
Risperidone	4.9	^{3}H-NMSP	Besnard et al. (2012)	60.6	^{3}H-SCH23390	Besnard et al. (2012)	5.2	^{3}H-nemonapride	Seeman (1995)	18.6	^{3}H-NMSP	Besnard et al. (2012)	16	^{3}H-SCH23390	Besnard et al. (2012)
SCH 23390	2145	^{3}H-domperidone	Grandy et al. (1989)	0.35	^{3}H-SCH23390	Sunahara et al. (1991)	>10,000	^{3}H-nemonapride	Neumeyer et al. (2003)	3560	^{3}H-spiperone	Van Tol et al. (1991)	0.3	^{3}H-SCH23390	Sunahara et al. (1991)
Spiperone	0.125	^{3}H-domperidone	Grandy et al. (1989)	577	^{3}H-SCH23390	Sunahara et al. (1991), Toll et al. (1998)	0.275	^{3}H-spiperone	Cussac et al. (2000), Neumeyer et al. (2003)	4	^{3}H-spiperone	Tang et al. (1994)	4500	^{3}H-SCH23390	Sunahara et al. (1991)
Thioridazine	10	^{3}H-NMSP	Grandy et al. (1989), Besnard et al. (2012)	89	^{3}H-SCH23390	Besnard et al. (2012)	5.2	^{3}H-spiperone	Seeman (1995)	17	^{3}H-NMSP	Besnard et al. (2012)	216	^{3}H-SCH23390	Besnard et al. (2012)
Trifluoperazine	1.3	^{3}H-NMSP	Kroeze et al. (2003)	740*	^{3}H-dopamine	Burt et al. (1976)				44	^{3}H-spiperone	Seeman et al. (1997), Besnard et al. (2012)			

Unmarked, human receptor. *, calf receptor. **, rat receptor.
[1]Most values and references from the PDSP K_I database (https://pdsp.unc.edu/databases/pdsp.php) except for Seeman (1995) and Lawler et al. (1999).

(GBM) cell line survival, the D_2R was also identified (Li et al., 2014). Inhibition of D_2R signaling with shRNA, short-interfering (si)RNA, and several antagonists (i.e., spiperone, haloperidol, risperidone, and L-741,626) reduced cell viability, proliferation, and clonogenicity in U87MG glioblastoma cells. To our knowledge, this was the only study to show that *DRD2* knockdown reduces cell viability and tumor growth.

D_2R Antagonists Reduce Cell Proliferation and Induce Apoptosis In Vitro. During the past 20 years, other studies also have identified D_2R antagonists as potential anticancer therapeutics through in vitro studies utilizing cell lines and patient samples (Table 4). Phenothiazines, most notably thioridazine, have been suggested as anticancer therapeutics more often than other chemotypes, but haloperidol, pimozide, and olanzapine also have been studied. These compounds have been shown to reduce cell viability, induce apoptosis, cause necrotic cell death, induce cell-cycle arrest, and alter protease activity (Fig. 1). This anticancer activity is apparent in a broad range of cancer types, including gender-specific (Kang et al., 2012; Mao et al., 2015; Park et al., 2016; Ranjan and Srivastava, 2016; Ranjan et al., 2016; Zhou et al., 2016), pancreatic (Ranjan and Srivastava, 2016), nervous system (Gil-Ad et al., 2004; Daley et al., 2005; Levkovitz et al., 2005; Shin et al., 2012, 2013; Li et al., 2014; Karpel-Massler et al., 2015), blood (Zhelev et al., 2004), oral (Choi et al., 2014), lung (Yue et al., 2016), gastric (Mu et al., 2014), and renal (Min et al., 2014) cancers, among others (Levkovitz et al., 2005; Nagel et al., 2012). Typical in vitro cell viability assay IC_{50} values for D_2R antagonists range from 5 to 20 μM, yet D_2R antagonists appear to be only modestly selective for cancer cells. Fibroblasts were less sensitive to pimozide treatment than five different pancreatic cancer cell lines, but there was a trivial difference in IC_{50} (2-fold selectivity, 10 vs. 20 μM) (Jandaghi et al., 2016). Astrocytic cell lines were also less sensitive to haloperidol compared with GBM cells (Li et al., 2014). These concentrations exceed the known maximum tolerated plasma concentrations in humans (Table 5) and suggest a narrow therapeutic window or even dose-limiting toxicity if applied to clinical use. In most cases, cytotoxic concentrations of these compounds are much higher (>100-fold) than would be expected for a D_2R-based mechanism, as determined from D_2R receptor affinity (Table 3). It is possible that this is owing to differences in receptor environment or functional partners, but it is also important to consider other mechanisms, especially because of the multiple targets that high concentrations of these drugs might engage (Besnard et al., 2012).

In Vivo Models of Cancer Suggest Efficacy of D_2R Antagonism. Animal models of cancer have suggested that D_2R antagonists might have chemotherapeutic utility (Table 6). Authors have reported significant reductions in tumor growth with D_2R antagonist treatment in gastric, glial, ovarian, medulloblastoma, oral, lung, pancreatic, prostate, and breast cancer xenograft models. Many of these studies observed evidence of Akt-signaling inhibition and/or alterations in autophagic flux in vivo. In an OVCAR-3 murine xenograft model, 10 mg/kg of thioridazine, trifluoperazine, or chlorpromazine reduced tumor growth, but an equivalent dose of fluphenazine was found to be toxic to the animals (Choi et al., 2008), again suggesting a narrow therapeutic window. A dose of 300 μg/day thioridazine or 400 μg/day mepazine reduced tumor size by half in OCI-Ly10 but not in Su-DHL-6 xenograft models (Nagel et al., 2012). These doses led to compound plasma levels of 200 ng/ml, well below the achievable plasma level of 2000 ng/ml in humans.

In summary, many animal studies have suggested that D_2R antagonists are efficacious in reducing tumor size and prolonging survival in xenograft models. In general, plasma and tumor drug concentrations were not quantified, but they may be expected to be well above selective concentrations. When measured in one study, plasma levels were, however, less than those achievable in human patients (Table 5) (Nagel et al., 2012). Unfortunately, toxicity of some compounds was observed. Therefore, D_2 receptor involvement is difficult to ascertain solely on the basis of pharmacological data. Ideally, such findings would be corroborated by studies that employed genetic methods to identify a target. To our knowledge, only one study probed the role of *DRD2* in a xenograft model in this way. In this study, a doxycycline-inducible *DRD2* knockout in U87MG intracranial xenografts prevented tumor growth in Nu/Nu mice, providing strong support for a role of D_2R in cancer growth (Li et al., 2014). Although most of these studies were carried out in the context of immunodeficient mice, it is tempting to speculate on the effects that D_2R modulators may have on the immune system through both indirect and direct means (i.e., through psychoactive effects or through direct interaction with immune cells).

D_2R Antagonists Are Associated with Anti–Cancer Stem Cell Activity. D_2R expression is also implicated in stem-like cells [cancer stem cells (CSCs)], hypothesized slow-cycling cells that promote tumor growth, chemoresistance, and metastasis. One in silico study using the Connectivity Map identified phenothiazines, notably trifluoperazine, as potential therapeutic agents capable of reversing stem-like gene expression profiles (Yeh et al., 2012). Trifluoperazine concentration-dependently induced apoptosis in a patient-derived, gefitinib-resistant lung cancer cell line, and reduced clonogenicity in a number of other patient-derived lines, regardless of epidermal growth factor receptor (EGFR) status. In a green fluorescent protein (GFP) reporter-based screen for Oct4 and Sox2 in human neoplastic pluripotent stem cells (hnPSCs), thioridazine appeared to target CSCs with an EC_{50} of 7 μM; prochlorperazine and fluphenazine were also identified, but not further characterized in this work (Sachlos et al., 2012). D_2R antagonists, including thioridazine (10 μM), reduced cell number and colony-forming units in AML samples and hPSCs (Sachlos et al., 2012). This work was the first to conclude that D_2R activity contributes to the survival and function of CSCs and employed both agonists and antagonists to examine this possibility. In glioblastoma CSCs, similar results were seen for the D_2R functionally selective, partial agonist aripiprazole (10 μM) (Suzuki et al., 2016) as well as the D_2R antagonists thioridazine and trifluoperazine and the selective D_4 antagonists PNU 96415E and L-741,742 (Dolma et al., 2016). Taken together, all of these results suggest that D_2R is expressed in CSCs and may impact stemness.

D_2R Receptor Signaling Mechanisms and Cancer Cell Growth

STAT and RTK Signaling. Signal transducer and activator of transcription (STAT) proteins are attractive therapeutic targets because of their role in cellular proliferation and angiogenesis. In a screen for potential STAT5 inhibitors using chronic myelogenous leukemia cell lines, the D_2R antagonist

TABLE 4
D_2 ligand IC_{50} values in cell culture

Compound	IC_{50} (μM)	Experiment Type	Cell Line	Source	Note
Aripiprazole	10–100	Trypan blue	PANC-1, PSN-1, A549	Ikai et al. (2016)	
Chlorpromazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	Found autophagy induction
Chlorpromazine	4.8–14.5	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Chlorpromazine	8	CellTiter-Blue	KOPT-K1	Gutierrez et al. (2014)	
Chlorpromazine	10	CCK-8	U87 MG	Shin et al. (2013)	
Chlorpromazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	
Fluphenazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Fluphenazine	3.9–7.9	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Haloperidol	>25	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Haloperidol	5–15	Clonogenicity	LN18, U87MG, T98G	Li et al. (2014)	
L-741,742	≥50	MTT	BJ, U2OS, Daoy	Dolma et al. (2016)	Cancer stem cell selective (8-fold selectivity)
L-741,742	1.56	MTT	G380	Dolma et al. (2016)	
Olanzapine	25–79.9	MTT	U87MG, A172, SC38, SC40	Karpel-Massler et al. (2015)	
Penfluridol	~5	MTT	MDA-MB-231, HCC-1806, 4T1	Ranjan and Srivastava (2016)	
Penfluridol	3, 4, 5	MTT	Panc-1, AsPC-1, BxPC-3	Ranjan and Srivastava (2016)	
Perphenazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Perphenazine	7	CellTiter-Blue	KOPT-K1	Gutierrez et al. 2014)	
Pimozide	7–15	Sulforhodamine B	Panc-1, CFPAC-1, Capan-1, BxPC-3, MiaPaCa-2	Jandaghi et al. (2016)	
Pimozide	~10	MTT	LNCaP, PC3M, 22RV1	Zhou et al. (2016)	
Pnu 96415e	>50	MTT	BJ, U2OS, Daoy	Dolma et al. (2016)	Cancer stem cell selective (eightfold selectivity)
Pnu 96415e	1.56	MTT	G380	Dolma et al. (2016)	
Prochlorperazine	>10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Thioridazine	5–10	Crystal violet	NCI-N87, AGS	Mu et al. (2014)	
Thioridazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	
Thioridazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Thioridazine	12.5–17.5	EZ-Cy Tox	KB, KBV20C	Choi et al. (2014)	
Thioridazine	3.8–6.7	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Thioridazine	11.2–15.1	Neutral red/ alamarBlue	C6, SHSY-5Y	Gil-Ad et al. (2004)	
Thioridazine	41.3	Neutral red/ alamarBlue	primary mouse brain	Gil-Ad et al. (2004)	
Thioridazine	~15	MTT	HeLa, C33A, Caski, HEC-1-A, KLE	Byun et al. (2012)	
Thioridazine			SiHa	Mao et al. (2015)	
Thioridazine		MTT	ISK, KLE	Meng et al. (2016)	Slowed growth over 96 h at 10 μm
Thioridazine				Min et al. (2014)	Sensitized to TRAIL at 10 μm
Thioridazine	~15	MTT	NCI-N87, AGS	Mu et al. (2014)	
Thioridazine	~10	MTT	ABC-DLBCL lines (HBL-1, Ocl-Ly3, U2932, TMD8)	Nagel et al. (2012)	
Thioridazine	>10	MTT	GCB-DLBCL lines (BJAB, Su-DHL-6, Su-DHL-4)	Nagel et al. (2012)	
Thioridazine	20	MTT	SKOV-3	Rho et al. (2011)	
Thioridazine	1–10	CFU	AML blasts	Sachlos et al. (2012)	Also reduces stemness
Thioridazine	10–20	Flow cytometry (Annexin V/PI)	4T1	Yin et al. (2015)	
Thioridazine	~10	MTT	NCI-H1299, 95-D	Yue et al. (2016)	
Trifluoperazine	>10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Trifluoperazine	4.3–7.7	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Trifluoperazine	~7	MTT	MDA-MB-231, BT549	Park et al. (2016)	
Trifluoperazine	10–15	MTT	Ca922	Wu et al. (2016)	
Trifluoperazine	>10	MTT	A549, H1975	Yeh et al. (2012)	
Trifluoperazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	
Trifluoperazine	5–10	MTT	H69, U1285, U-1906, U-2020	Zong et al. (2014)	

TABLE 5
Tolerated human plasma levels of selected D_2 antagonists

Compound	[C] plasma in humans (nM)		Source
	Max	Min	
Thioridazine	2699	270	Smith et al. (1984)
Chlorpromazine	1548	101	Chetty et al. (1999)
Pimozide	32	2	Kerbusch et al. (1997)
Olanzapine	40	31	Kassahun et al. (1997)
Haloperidol	67	11	Froemming et al. (1989)
Clozapine	4525	1007	Guitton et al. (1998)

pimozide (5–10 μM) decreased STAT5 phosphorylation and function, even downstream of potent oncogenic activation (Nelson et al., 2011). Moreover, pimozide inhibited IL-6-induced growth and migration via inhibition of STAT3 in prostate cancer cells (Zhou et al., 2016). It is unknown if other D_2R antagonists inhibit STAT directly, but they reduce proneoplastic receptor tyrosine kinase (RTK) signaling upstream of Janus kinase (JAK)/STAT. The D_2R agonists quinpirole (10 μM) and pramipexole (10 μM) both increased phosphorylation of extracellular signal-regulated kinases (ERK) and RTK EGFR in a D_2R-dependent manner (Yoon and Baik, 2013). Antagonism with thioridazine reduced vascular EGFR (VEGFR) phosphorylation and VEGF availability (Park et al., 2014). These studies suggest an RTK/JAK/STAT mechanism or downstream effect of D_2R antagonists and a possible role for D_2R in proneoplastic EGFR signaling.

Wnt. The wingless/integrated (Wnt) pathway affects development, carcinogenesis, and stem-like behavior, and is reportedly inhibited by D_2R antagonists. In a patient-derived lung cancer cell line, trifluoperazine concentration-dependently inhibited T-cell factor–mediated transcription (Yeh et al., 2012), with the decreases in Wnt signaling being concomitant with the induction of cytotoxicity. Such findings are supported by an in silico docking and network analysis study identifying the Wnt pathway protein glycogen synthase kinase (GSK)3β as potentially affected by phenothiazine treatment (Qi and Ding, 2013). Spiperone (10 μM) had similar effects, but these were not mediated by D_2R, serotonin, or $\sigma_{1/2}$ receptor activity by comparison with selective receptor ligands but may have involved intracellular calcium signaling and protein kinase C (PKC) (Lu and Carson, 2009). Furthermore, D_2R and Wnt5a coimmunoprecipitated from HEK293T cells with a K_I of 165 nM for competition with [^{3}H]-spiperone, suggesting a possible direct interaction (Yoon et al., 2011). The quinpirole-induced upregulation of Wnt pathway protein Dvl-3 induces ERK activation in mesencephalic neuronal culture but did not occur using cells from $D_2R^{-/-}$ mice (Yoon et al., 2011). These data suggest that the D_2R may interact with the Wnt pathway in neuronal cells and that D_2R antagonists can decrease Wnt signaling, but further studies are needed to see if this is more broadly applicable to the malignant phenotype.

PI3K. The PI3K/Akt pathway, a critical regulator of the cell cycle, has been suggested as a target pathway for D_2R antagonists in cancer-related cell lines. In Chinese hamster ovary (CHO) cells expressing the human D_2R, dopamine and quinelorane activated the PI3K pathway by increasing phospho-Akt (at both Ser-473 and Thr-308) and GSK-3β (at Ser-9) levels, with maximal effects at 10 μM (Mannoury la Cour et al., 2011). Pertussis toxin, as well as D_2R antagonists, blocked this, suggesting a dependence on D_2R G protein signaling. When receptor internalization was blocked with phenylarsine oxide, phosphorylation levels were reduced by half. Likewise, disruption of cholesterol-rich lipid rafts with methyl-β-cyclodextrin inhibited phosphorylation. These latter data suggest that both G-protein and β-arrestin signaling are important. Increased Akt phosphorylation was PKC- and calmodulin-dependent, and GSK-3β phosphorylation was attributable, at least in part, to Akt activity. Thus, there is the potential for these mechanisms to affect cancer cell growth, proliferation, and metabolism via Akt downstream effectors, including transcription factors (like FOXO). In vivo, 25 mg/kg thioridazine given every third day to 2774-xenografted (ovarian cancer) nude mice reduced phosphorylation levels of PI3K, Akt, pyruvate dehydrogenase kinase 1 (PDK1), and mammalian target of rapamycin (mTOR) (Park et al., 2014). In normal rat brain, however, D_2R antagonist raclopride (3 mg/kg per day) enhanced phosphorylation at both Thr^{308} and Ser^{473} of Akt, which indicates activation, but did not alter total Akt protein levels. In the same model, agonist quinpirole reduced phosphorylation (Sutton and Rushlow, 2012). In normal brain, Akt phosphorylation is reduced by D_2-receptor activation in a β-arrestin-2 (βArr2)-mediated manner involving a complex with PP2A (Sotnikova et al., 2005). Antagonism may increase the overall level of Akt phosphorylation or block cell sensitivity to βArr2-mediated Akt regulation (Beaulieu et al., 2004). $D_2R^{-/-}$ mouse striatal lysates have increased Akt phosphorylation at Thr-308 both basally and in response to amphetamine (3 mg/kg) challenge (Beaulieu et al., 2007). Overall, it appears PI3K signaling is increased by D_2R agonists but reduced by D_2R antagonists in malignant tissues, whereas the opposite may be true in normal tissues.

Thioridazine (15 μM) induced apoptosis and inhibited the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway in endometrial and cervical cancer cell lines (Kang et al., 2012), and at similar concentrations had effects resembling PI3K/Akt inhibition (Rho et al., 2011), decreasing PI3K activity by 60%, inducing G1 arrest after 24-hour treatment, reducing cell viability by half at 48 hours, and inducing apoptosis. Phosphorylation of Akt, mTOR, and GSK-3β were also reduced by several antidopaminergic phenothiazine drugs at low micromolar concentrations in EGF-stimulated OVCAR-3 ovarian cancer cells, although the concentration-response relationship did not parallel D_2R affinity (Choi et al., 2008). PI3K activation was unaffected by these phenothiazines.

MAPK/ERK. The mitogen-activated protein kinase (MAPK)/ERK pathway, known to be involved in cancer cell survival and proliferation, was inhibited in U87MG and A172 glioma cell lines by four different D_2R antagonists, albeit at relatively high concentrations (spiperone and haloperidol at 5 μM, risperidone and L-741,626 at 10 μM) (Li et al., 2014). MAPK8 and MAPK10 were also identified as potential targets by a correlational in silico docking and network analysis study of phenothiazines, including chlorpromazine, fluphenazine, and trifluoperazine (Qi and Ding, 2013). This may involve a cascade wherein interaction with peroxisome proliferator-activated receptors γ affects MAPK8 status, leading to a protein kinase-modulated alteration of activity in downstream effectors cyclin-dependent kinase 2 and GSK3β (see section on Wnt signaling). In normal rat and mouse brain slices, the D_2 agonist quinpirole (60 μM) increased MAPK and cAMP response element-binding protein phosphorylation,

TABLE 6
D_2 antagonist efficacy in animal studies

Compound	Dose (mg/kg)	Timing	Model	Efficacy	Source	Note
Bromocriptine	1 to 2	QD	MNNG-induced Wistar rats (gastric cancer)	~2.5-Fold increase in tumor number	Iishi et al. (1992)	Agonist
Chlorpromazine	10	QD×5	OVCAR-3 Xenograft, nude mice	64% Tumor growth suppression	Choi et al. (2008)	
Chlorpromazine	20	QD	U87MG Xenograft	43.5% Inhibition of tumor growth	Shin et al. (2013)	
Fluphenazine	10	QD×5	OVCAR-3 Xenograft, nude mice	Toxicity - ND	Choi et al. (2008)	
Haloperidol	10	QD	MiaPaCa-2 Xenograft in NSG mice	~50% Decrease in tumor mass	Jandaghi et al. (2016)	
Haloperidol	10	QD	U87MG Xenograft	No significant reductions in tumor size or survival	Li et al. (2014)	Synergy with AG1478
Imipramine	40	QD	GRLp53het Mice	2-Fold increase in survival time over control	Shchors et al. (2015)	Cooperated with ticlopidine to enhance autophagy and increase survival
L-741,742	20	QD	G362 Xenograft, flank and intracranial	40.9% Reduction in tumor mass, prolonged survival	Dolma et al. (2016)	Synergy with TMZ
Penfluridol	10	QD	4T1 Orthotopic mammary xenografts, female Balb/c mice	49% Reduction in tumor size	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	4T1-luc Intracardiac metastasis model, female Balb/c	90% Reduction in brain fluorescence from luciferase reporter	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	4T1 Intracranial xenograft	~33% Reduction in brain fluorescence from luciferase reporter	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	BxPC-3 Xenografts (subcutaneous) in athymic nude mice	~50% Reduction in tumor volume at day 27	Ranjan and Srivastava (2016)	
Perphenazine	10	QD	hTALL2 Cells in NSG mice	~33% Reduction in luciferase bioluminescence	Gutierrez et al. (2014)	
Pnu 96415e	20	QD	G362 Xenograft, flank	44.3% Reduction in tumor mass	Dolma et al. (2016)	
Quinpirole	10	QD	LLC1 Xenograft	~66% Reduction in bioluminescence	Hoeppner et al. (2015)	Agonist
Thioridazine	10	QD×5	OVCAR-3 Xenograft, nude mice	26% Tumor growth suppression	Choi et al. (2008)	
Thioridazine	Pretreatment of cells		NCI-N87 Xenograft	Slight, insignificant reductions in tumor size or survival	Mu et al. (2014)	Pretreatment with 5 μm thioridazine
Thioridazine	300 μg/animal	QD	Ocl-Ly10 Xenograft, nude mice		Nagel et al. (2012)	
Thioridazine	25	QD	2774 Xenografts, nude mice	>50% Reduction in tumor size and volume, significantly reduced Ki67 staining	Park et al. (2014)	Oral delivery
Thioridazine	32	QD	4T1 Xenograft in BALB/c	55% Reduction in tumor volume	Yin et al. (2015)	
Thioridazine	Pretreatment of cells		NCI-H1299	~50% Reduction in tumor size at day 47	Yue et al. (2016)	
Trifluoperazine	10	2×/wk	MDA-MB-231 Xenografts, nude mice	~50% Reduction in tumor volume at day 33	Park et al. (2016)	
Trifluoperazine	5	QD	CL97 Tail vein injection NOD/SCID	Significant reduction in bioluminescence/tumor size	Yeh et al. (2012)	
Trifluoperazine	10	QD×5	OVCAR-3 Xenograft, nude mice	46% Tumor growth suppression	Choi et al. (2008)	

with effects blocked by the D_2 antagonist eticlopride (40 μM), the calcium chelator BAPTA-AM, or the PKC antagonist Go6976 (Yan et al., 1999). Although these investigators did not directly assay G protein activity, they hypothesized a role for $G\alpha_q$ activation (Yan et al., 1999), although the D_2-like receptors normally are not considered to couple readily to this α-subunit. Owing to the heterogeneous nature of the system and use of healthy tissue, these findings may or may not have any relationship to the behavior of cancer cells exposed to ligands that modulate D_2R function.

Calcium Signaling. D_2R signaling and antagonist treatments both alter calcium signaling. Wolfe and Morris (1999) found that both the long and short D_2R isoforms interacted with $G_{\alpha o}$ to reduce high-voltage-activated calcium channel activity. In wild-type astroglia, dopamine signaling is capable of both increasing and reducing intracellular calcium levels in a manner dependent on local neural type in brain slices (Jennings et al., 2017). Dopamine D_2/D_3 receptors were involved in the negative regulation of Ca^{2+} in this study.

The calcium channel blocker bepridil and the D_2R antagonist triflupromazine had similar effects on PI3K signaling through FOXO3 in MDA-MB-231 breast cancer cells (Park et al., 2016). FOXO3 activity was required to reduce colony formation with both trifluoperazine and bepridil, and FOXO3-regulated proteins D_2R, KLF-5, and c-Myc were downregulated by treatment with either drug. In vivo, 10 mg/kg trifluoperazine or bepridil three times a week significantly reduced tumor volume of MDA-MB-231 xenografts in female athymic (nu/nu) mice (Park et al., 2016). A calmodulin mechanism was posited for both compounds but not explored experimentally.

In pancreatic cancer lines MiaPaCa-2 and Panc-1, 10 μM pimozide, or L-741,626 increased intracellular calcium levels sharply within seconds of treatment and concentration-dependently increased phospho–protein kinase R–like endoplasmic reticulum kinase (PERK), suggesting an increase in endoplasmic reticulum stress (Jandaghi et al., 2016). PKA phosphorylation activity was also modestly increased. Caspase activity upon treatment with pimozide was reduced by around 25% when ATF4 was silenced with shRNA, further supporting the involvement of the unfolded protein response. Similar results were found for haloperidol, except IC_{50} values were increased and fibroblasts seemed even more resistant. Overall, it appears that multiple chemotypes of D_2R antagonists can alter intracellular calcium levels and initiate cellular stress in cancer cells.

Autophagy May Be Affected by D_2 Antagonists. Numerous studies have suggested that D_2R antagonists are able to induce autophagic cell death in the context of in vitro and in vivo studies of cancer. One trifluoperazine derivative, A4, increased reactive oxygen species (ROS), DNA damage, and autophagic cell death, while also causing apoptosis and activating AMP-activated protein kinase K (AMPK) (Wu et al., 2016). AMPK phosphorylation increases were also seen in D_2R antagonist-treated GBM stem cell cultures (Cheng et al., 2015). In SH-SY-5Y neuroblastoma cells, sertindole, pimozide, and trifluoperazine were identified as autophagy-inducing agents by a large-scale fluorescence-based screen (Shin et al., 2012). Increases in GFP-LC3 puncta were sertindole concentration- and time- dependent; autophagosome formation was also verified by electron microscopy. LC3 cleavage was responsive to GFP 3-methyladenine, suggesting autophagic induction was partially regulated by the PI3K pathway. Conditional siRNA knockdown of the essential autophagic protein, ATG5, reduced autophagosome formation, enhanced cell viability, and reduced LC3 cleavage under treatment with 10 μM sertindole. A fluorescence assay that included ROS scavengers indicated a partial role for ROS in the cytotoxicity of sertindole. Similar results have been reported in glioma cell lines (Shin et al., 2013; Cheng et al., 2015). Although autophagy can contribute to D_2 antagonist-mediated cell death, D_2 activity does not appear to be involved in this mechanism since thioridazine reduced D_2R protein levels and increased autophagy, whereas trifluoperazine reduced D_2R protein levels and did not increase autophagy at the same concentrations.

Lipid Synthesis and Trafficking Is Altered by D_2R Antagonist Treatment. An early study reported that chlorpromazine (10 μM) inhibited both sphingomyelinase activity and esterification of cholesterol in human fibroblasts in a manner comparable to 10 μM W-7, a known calmodulin antagonist (Masson et al., 1992). Chlorpromazine treatment resulted in accumulation of unesterified cholesterol in lysosomal vacuoles reminiscent of a Niemann-Pick type C (NPC) lipidosis phenotype (Masson et al., 1992). Similar results were seen with 10–50 μM haloperidol, and concomitant insulin receptor signaling inhibition was reversed by cholesterol add-back, suggesting lipid raft disruption (Sanchez-Wandelmer et al., 2010).

Other antipsychotics like haloperidol (10 μM) and clozapine (30 μM) increased cholesterol and fatty acid synthesis enzyme mRNA by 2- to 4-fold in GaMg glioma cells at 5–10 hours (Ferno et al., 2006). Sterol regulatory element-binding proteins (SREBP)-1 and SREBP-2, sterol-responsive transcription factors that regulate these genes, were upregulated at the protein level, supporting the idea that antipsychotic treatment may upregulate lipogenesis via SREBP signaling. Cholesterol-related mRNAs, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), apolipoprotein E (APOE), ATP binding cassette subfamily A member 1 (ABCA1), liver X receptors (LXR)α/β, and NPC1/2, were increased after 24- to 48-hour treatment with clozapine (25 μM), haloperidol (10 μM), olanzapine (10 μM), or imipramine in GaMg cells (Vik-Mo et al., 2009). Protein levels of apoE also increased in GaMg and HepG2 human hepatocellular carcinoma cells. Message-level increases were more striking in glial cell cultures, suggesting the activation of LXR and its downstream targets may occur as an effect of earlier SREBP-modulated lipogenesis within the cell (Ferno et al., 2006). Lipogenesis and adequate cholesterol stores are essential for cancer cell survival, particularly in the case of gliomas, which are highly sensitive to exogenous cholesterol levels and LXR activity (Villa et al., 2016).

Although haloperidol and pimozide treatment (10 μM) slightly increased the expression of some SREBP-responsive genes, they also disrupted cholesterol trafficking, causing intracellular accumulation of unesterified cholesterol in intracellular puncta in CHO-7 cells (Kristiana et al., 2010). Despite increases in active SREBP-2, cholesterol synthesis was ablated under treatment with these compounds. Aripiprazole, clozapine, quetiapine (all 10 μM), olanzapine, risperidone, and ziprasidone (25 μM) showed similar behavior, suggesting that the effect may be mediated by D_2R or another common target of these compounds. Kristiana et al. (2010) posited that the intracellular trafficking of cholesterol was disrupted by these drugs, inhibiting SREBP cleavage-activating

protein (SCAP) activation of SREBP and sterol *O*-acyltransferase 1 (SOAT-1) esterification of cholesterol. Likewise, 10–50 μM haloperidol reduces biosynthesis of cholesterol in SH-SY-5Y cells while generating a buildup of sterol precursors (Sanchez-Wandelmer et al., 2010). Risperidone, ziprasidone, and clozapine (5–25 μM) also induced buildup of sterol intermediates in HepG2 cells (Canfran-Duque et al., 2013).

Clearly, numerous chemotypes of D_2R antagonists can reduce cellular cholesterol levels, disrupt lipid rafts, and alter lipid trafficking. These effects, however, have not been shown to be the cause of D_2R antagonist-induced cytotoxicity; it is possible that lipid alterations result from cellular coping mechanisms for other types of stress, such as ROS or autophagic stress. Indeed, these lipid phenotypes indicate that cancer cells treated with these compounds behave as though they are lipid-starved and frustrated in their attempts to synthesize more. One final point should be noted—many (but not all) of the D_2-like antagonists used clinically can cause a metabolic syndrome that can include hyperlipidemia (Hirsch et al., 2017; Hoffman, 2017), yet the latter effects require chronic use of the antipsychotics, and the drug concentrations in human tissue are far lower than those causing anticancer effects in vitro or in animals. These factors suggest that different mechanisms probably are involved.

D_2R Antagonists May Interact Positively with Other Compounds to Increase Their Anticancer Efficacy. Studies also indicate that D_2R antagonists can be additive with common chemotherapeutics. Aripriprazole sensitized CSC-enriched cultures to gemcitabine, 5-FU, and cisplatin treatment in an additive manner (Suzuki et al., 2016). Likewise, the proapoptotic effects of trifluoperazine were synergistic with cisplatin (10 μM) and gefitinib (2.5–10 μM) in a patient-derived lung cancer cell line (Yeh et al., 2012). Tumor volume and weight of G362 GBM xenografts were decreased in mice treated with 20 mg/kg of either PNU 96415E or L-741,742 over control, though the difference in size was not large (Dolma et al., 2016). L-741,742 treatment on its own failed to improve survival of xenografted mice, but survival increased under cotreatment with temozolomide over treatment with temozolomide alone. Likewise, thioridazine increased the efficacy of AraC in leukemia (Dolma et al., 2016), and cisplatin or paclitaxel in ovarian cancer (Rho et al., 2011). In treatment-resistant endometrial cancer cell lines ISK and KLE, combination treatment with 20 μM medroxyprogesterone acetate and 10 μM thioridazine reduced cell viability by half after 4 days (Meng et al., 2016). Such observations could potentially be explained by inhibition of P-glycoprotein or other efflux pumps associated with drug resistance, as suggested by the fact that thioridazine sensitizes chemoresistant oral squamous cancer cells (KBV20C) to vinblastine owing to inhibited P-glycoprotein efflux (Choi et al., 2014). Likewise, ABCG2-mediated chemoresistance in MDR cells is reduced by 10 μM D_3 antagonists PG01037, NGB 2904, SB27 7011A, and U99194 (Hussein et al., 2017). Hussein et al. (2018) later reported that cariprazine (which they termed a D_2/D_3 partial agonist) had similar effects and suggested it might be repurposed for cancer chemotherapy. The concentrations required, however, were $\geq$1 μM, much higher than found clinically with maximal doses of the cariprazine, a drug that also has active metabolites that accumulate at even higher levels (Nakamura et al., 2016). These facts suggest that repurposing of the parent molecule might be problematic, and that the reported actions might not be via D_2 or D_3 receptors.

Critical Interpretation and Future Directions

As the literature currently stands, evidence is suggestive, but by no means conclusive, of an anticancer role for D_2R antagonists. Correlative studies of patients with schizophrenia and PD, case studies of cancer patients under concomitant antipsychotic therapy, and repeated hits by unbiased screens support the notion that D_2R may have a significant role in cancer development and may be a reasonable therapeutic target. Also, D_2R antagonists of varying chemotypes have anticancer activity both in vitro and in vivo, where they induce apoptosis, autophagic cell death, and cell cycle arrest (Fig. 1). In some studies, they also induce CSC differentiation and/or disrupt cholesterol trafficking and synthesis. Such effects are favorable for anticancer therapies, especially since these compounds are modestly selective for cancer cells over normal cell type controls of various lineages.

Yet, although these compounds have effects and can affect many signaling pathways (Figure 3), the role of the D_2R itself is still unclear. One major factor is that invariably the concentrations required to induce cytotoxicity are many orders of magnitude higher than the K_D for this receptor. At these concentrations, this class of drug has many off-target actions. As approved drugs, there is a great deal of data regarding pharmacokinetics, pharmacodynamics, and toxicity profiles, which when considered in the light of the modest selectivity in cell culture studies, suggests that it may be difficult to achieve circulating plasma levels sufficient for meaningful anticancer activity (Table 5). Maximal circulating levels are reported as concentrations of parent compound, although some of these compounds would also be present as active or inactive metabolites that may or may not have anticancer activities. Many D_2R antagonists also have profound side effects that included marked increase in serum prolactin, large increases in body weight and metabolic syndrome, neurologic side effects, and potentially fatal cardiac complications like torsades de pointes that results from QT prolongation. Although some of these are quite serious, they may be tolerable in patients with cancers that are unresponsive to other therapies, especially if the side effects are reversible. The question is whether there is an adequate therapeutic window and an adequate degree of efficacy.

Another issue arising from the high concentrations necessary for anticancer effects is that of target determination; it is far from clear that the D_2R is a valid anticancer target on the basis of pharmacological studies alone. Aside from the studies of Li et al. (2014) with GBM, there is little in vitro or in vivo data to suggest that alteration of D_2R levels can affect cell growth, viability, or response to D_2R antagonist treatment. Studies to determine the role of the D_2R will require both understanding of basic principles of pharmacology and the use of orthogonal approaches to decrease the likelihood of erroneous conclusions. Thus, if the D_2R is hypothesized to be the target by which an antipsychotic drug kills or inhibits cancer cell growth, then rigorous evidence must be provided to demonstrate that the receptor is both expressed on the cell type of interest and the principal target that needs to be engaged. Ideally a combination of approaches such as receptor binding assays, Western blot, immunosorting analysis, mRNA

quantification, molecular ablation, and the like are needed to provide a rigorous test of the underlying hypothesis. Without these types of data, assigning activity to a specific target is risky.

As an example, ONC201, a small-molecule inhibitor of TNF-related apoptosis-inducing ligand (TRAIL), reduced proliferation and viability in HCT116 gastric cancer cells (Allen et al., 2016) with antagonism of the D_2R as a major part of its activity. The mechanism was defined further by the suggestion by Leng et al. (2017), who hypothesized a D_5R modulation of the D_2R by direct experiments using the nanomolar affinity D_1/D_5 agonist SKF83959 (Lee et al., 2014a) and the nanomolar affinity D_2-like agonist cabergoline (Newman-Tancredi et al., 2002). They found that ligand concentrations ≥ 5 μM or higher were required to cause effects for these drugs. Despite the good selectivity of SKF83959, and to a lesser extent cabergoline, micromolar concentrations will engage many off-target effects as known for SKF83939 (Lee et al., 2014b). On the basis of the pharmacological principles underlying the current analysis, there should be significant skepticism about this proposed D_5R/D_2R mechanism. Shortly thereafter, it was shown that the cytotoxicity of ONC201 was not eliminated by D_2R knockdown or knockout (Kline et al., 2018). Moreover, in a preliminary clinical study against glioblastoma multiformae, ONC201 increased circulating prolactin by only 20% (Arrillaga-Romany et al., 2017), whereas known D_2 antagonists cause multifold increases, inconsistent with effects via the D_2R. Although they had shown that D_2R knockdown or knockout was not the primary mechanism for ONC201 (Kline et al., 2018), these investigators (Prabhu et al., 2019) use associations and correlations of expression data (without any direct assessment of pharmacology or signaling) to elaborate further on the D_5R-D_2R modulation suggested by Leng et al. (2017). A pharmacological analysis suggests that neither receptor is of primary importance.

In summary, we were attracted to this topic because it seemed like an excellent example of the potential for drug repurposing with a known target (i.e., D_2R) for which dozens of drugs are approved, and for which there are probably thousands of experimental compounds that already exist. If the D_2R is a viable target, such a wealth of compounds and data would be a very fertile field for study. Yet, our attempt at a critical view of the literature has altered our initial opinion, such that we believe it is probable that the actions of D_2R antagonists both in vivo and in vitro will not in most cases involve effects mediated primarily by the D_2 receptor. Indeed, novel phenothiazine derivatives have been shown to have many potential anticancer activities aside from the established activities with respect to calmodulin, dopamine receptors, and other known psychiatrically relevant targets. These include antioxidant ability, inhibition of tubulin polymerization, and inhibition of farnesyl transferase (Prinz et al., 2011; Baciu-Atudosie et al., 2012; Engwa et al., 2016; Ghinet et al., 2016). We recognize how our hypothesis, which runs counter to a voluminous literature, could be interpreted, but we believe it would be useful if this generates controversy that leads to hypothesis-driven studies using orthogonal approaches and varying structural series of D_2R antagonists. Such rigorous pharmacological evidence could help clarify many of the intrinsic issues. Whether our supposition is correct or not, the field will benefit from a clear resolution of these questions, and the knowledge might impact on the development of new therapeutic paradigms.

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Authorship Contributions

Performed data analysis: Weissenrieder, Mailman.

Wrote or contributed to the writing of the manuscript: Weissenrieder, Mailman, Neighbors, Hohl.

Note Added in Proof—Some references and footnote were accidentally not included in Table 3 in the Fast Forward version published April 18, 2019. Table 3 has now been corrected.

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