G-Protein–Coupled Receptor 30 and Estrogen Receptor-α Are Involved in the Proliferative Effects Induced by Atrazine in Ovarian Cancer Cells

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BACKGROUND: Atrazine, one of the most common pesticide contaminants, has been shown to up-regulate aromatase activity in certain estrogen-sensitive tumors without binding or activating the estrogen receptor (ER). Recent investigations have demonstrated that the orphan G-protein–coupled receptor 30 (GPR30), which is structurally unrelated to the ER, mediates rapid actions of 17β-estradiol and environmental estrogens.

OBJECTIVES: Given the ability of atrazine to exert estrogen-like activity in cancer cells, we evaluated the potential of atrazine to signal through GPR30 in stimulating biological responses in cancer cells.

METHODS AND RESULTS: Atrazine did not transactivate the endogenous ERs in different cancer cell contexts or chimeric proteins encoding the ERα and ERβ hormone-binding domain in gene reporter assays. Moreover, atrazine neither regulated the expression of ERα nor stimulated aromatase activity. Interestingly, atrazine induced extracellular signal-regulated kinase (ERK) phosphorylation and the expression of estrogen target genes. Using specific signaling inhibitors and gene silencing, we demonstrated that atrazine stimulated the proliferation of ovarian cancer cells through the GPR30–epidermal growth factor receptor transduction pathway and the involvement of ERα.

CONCLUSIONS: Our results indicate a novel mechanism through which atrazine may exert relevant biological effects in cancer cells. On the basis of the present data, atrazine should be included among the environmental contaminants potentially able to signal via GPR30 in eliciting estrogenic action.

KEY WORDS: 17β-estradiol, atrazine, estrogen receptor, GPR30, ovarian cancer cells. Environ Health Perspect 116:1648–1655 (2008). doi:10.1289/ehp.111297 available via http://dx.doi.org/ [Online 22 July 2008]

Atrazine belongs to the 2-chloro-s-triazine family of herbicides (Figure 1) and is the most common pesticide contaminant of groundwater and surface water (Fenelon and Moore 1998; Kolpin et al. 1998; Lode et al. 1995; Miller et al. 2000; Müller et al. 1997; Solomon et al. 1996; Thurman and Cromwell 2000). Among the endocrine-disrupting effects, atrazine interferes with androgen- and estrogen-mediated processes (Babic-Gojmerac et al. 1989; Cooper et al. 1999, 2000; Cummings et al. 2000; Friedmann 2002; Kniewald et al. 1979, 1995; Narotsky et al. 2001; Shafer et al. 1999; Simic et al. 1991; Stoker et al. 1999, 2000). The interference of atrazine with androgen and estrogen action does not occur by direct agonism or antagonism of cognate receptors for these steroids as shown by binding affinity studies (Roberge et al. 2004; Tennant et al. 1994a, 1994b). In this respect, previous investigations have suggested that atrazine reduces androgen synthesis and action (Babic-Gojmerac et al. 1989; Kniewald et al. 1979, 1980, 1995; Simic et al. 1991) and stimulates estrogen production (Crain et al. 1997; Heneweer et al. 2004; Keller and McClellan-Green 2004; Sanderson et al. 2000, 2001, 2002; Spano et al. 2004). The latter ability is exerted through at least two mechanisms that converge on increasing aromatase expression and activity. First, inhibiting phosphodiesterase, atrazine up-regulates cAMP, which induces the expression of SF-1, an important regulator of the PII promoter of aromatase gene CYP19. The enhanced transcription of the aromatase gene increases both enzymatic activity of aromatase and estrogen production (Heneweer et al. 2004; Lehmann et al. 2005; Motinaga et al. 2004; Roberge et al. 2004; Sanderson et al. 2000, 2001). Next, atrazine binds to SF-1 and facilitates the recruitment of this factor to the PII promoter of the aromatase gene, further stimulating the biological effects described above (Fan et al. 2007a, 2007b).

Epidemiologic studies have associated long-term exposure to triazine herbicides with increased risk of ovarian cancer in female farm workers in Italy (Donna et al. 1989) and breast cancer in the general population of Kentucky in the United States (Kettles et al. 1997). In addition, atrazine leads to tumor development in the mammary gland and reproductive organs of female F344 rats (Pinter et al. 1994), whereas in Sprague-Dawley rats it causes an earlier onset of mammary and pituitary tumors (Wetzel et al. 1994), a typical response to exogenously administered estrogens (Brawer and Sonnenschein 1975).

Given the potential ability of atrazine to interfere with reproduction and to cause cancer, the European Union banned its use. However, the U.S. Environmental Protection Agency has approved the use of atrazine because of the lack of a clear association between the levels of exposure and cancer incidence in pesticide applicators (Gammon et al. 2005; McElroy et al. 2007; Rusiecki et al. 2004; Sass and Colangelo 2006; Young et al. 2005).

Regarding the apparent estrogenic effects of atrazine, previous studies have demonstrated that triazine herbicides do not bind or activate the classical estrogen receptor (ER) (Connor et al. 1996; Tennant et al. 1994a, 1994b). In recent years, increasing evidence has demonstrated in different experimental models that steroid hormones, including estrogens, can exert rapid actions interacting with receptors located within or near the cell membrane (Falkenstein et al. 2000; Norman et al. 2004; Revelli et al. 1998). The importance of this signaling mechanism is becoming more widely recognized as steroid membrane receptors have been implicated in a large number of physiologic functions. Moreover, it has been suggested that nongenomic estrogen actions, like genomic ones, are susceptible to interference from environmental estrogens (Thomas 2000).

Of note, these compounds compete with [3H]17β-estradiol ([3H]E2) for binding to estrogen membrane receptors (Loomis and Thomas 2000) and exert agonist effects on nongenomic transduction pathways in different cell contexts (Loomis and Thomas 2000; Nadal et al. 2000; Ruchmann et al. 1988; Watson et al. 1999). However, the precise identity and function of many steroid membrane receptors are still controversial in terms of their specific molecular interactions with endogenous and environmental estrogens.

A seven-transmembrane receptor, G-protein–coupled receptor 30 (GPR30), which is structurally unrelated to the nuclear ER, has been recently shown to mediate rapid actions of estrogens (Filardo et al. 2002; Revankar et al. 2005). Recombinant GPR30 protein, produced in ER-negative HEK-293 cells, exhibited all the steroid binding and signaling...
characteristics of a functional estrogen membrane receptor (Thomas et al. 2005; Thomas and Dong 2006). Our studies and others have also demonstrated that GPR30 mediates the rapid response to E2 in a variety of estrogen-responsive cancer cells by activating the epidermal growth factor receptor (EGFR)–mitogen-activated protein kinase (MAPK) transduction pathway (Albanito et al. 2007; Bologa et al. 2006; Filardo et al. 2000; Maggiolini et al. 2004; Revankar et al. 2005; Thomas et al. 2005; Vivaquassa et al. 2006a, 2006b).

In the present study, for the first time we have demonstrated that atrazine stimulates gene expression and growth effects in estrogen-responsive ovarian cancer cells through GPR30 and the involvement of ERα. Moreover, we show that GPR30 mediates the stimulatory effects of atrazine in ER-negative SkBr3 breast cancer cells.

Materials and Methods

Reagents. We purchased atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3-triazine], 3β,17β-estradiol (E2), N,N′-[2-(p-bromocinnamylamino)ethyl]-5-isouquinolinesulfonamide dihydrochloride (H89), wortmannin (WM), and the involvement of ERα in mediating the rapid response to E2 in a variety of estrogen-responsive cancer cells through GPR30 and the involvement of ERα. Moreover, we show that GPR30 mediates the stimulatory effects of atrazine in ER-negative SkBr3 breast cancer cells.

Materials and Methods

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with 1 nM [2,4,6,7-3H]E2 (89 Ci/mmol; Amersham Biosciences) and increasing concentrations of unlabeled E2 or atrazine for 1 hr at 37°C in a humidified atmosphere of 95% air/5% CO2. After removal of the medium, cells were washed with ice-cold phosphate-buffered saline/0.1% methylcellulose twice, harvested by scraping and centrifugation, and lysed with 100% ethanol, 500 µL/60-mm dish, for 10 min at room temperature (Lee and Gorski 1996). We measured the radioactivity of extracts by liquid scintillation counting.

**Aromatase assay.** In subconfluent BG-1 or H295R cells, we measured aromatase activity in the cell culture medium by titrated water release using 0.5 µM [1β-3H(N)]androst-4-ene-3,17-dione (25.3 Ci/mmol; DuPont NEN, Boston, MA, USA) as a substrate (Lephart and Simpson 1991). The cells were treated in a six-well dish in culture medium in the presence of atrazine or DMSO for 40 hr. Values shown are mean ± SD of three independent experiments performed in triplicate.

Figure 2. ERα transactivation in BG-1 (A), MCF-7 (B), and Ishikawa (D) cells transfected with the ER luciferase reporter plasmid XETL (ERE-luc) and treated with 100 nM/L E2 or 1 µM/L atrazine (Atr), with and without 10 µM/L ER antagonist ICI. Luciferase activities were normalized to the internal transfection control, and values of cells receiving vehicle (–) were set as 1-fold induction, from which the activity induced by treatments was calculated. (D–F) SkBr3 cells were transfected with ER luciferase reporter gene XETL and ERα expression plasmid (D) and with Gal4 reporter gene (GK1) and the Gal4 fusion proteins encoding the HBD of ERα (GalERα; E) and or ERβ (GalERβ; F) and treated with 100 nM/L E2 or 1 µM/L atrazine, with and without 10 µM/L ICI. Values shown are mean ± SD of three independent experiments performed in triplicate. *p < 0.05 compared with vehicle.

**Figure 3.** mRNA expression and binding of ERα in BG-1 cells treated for 24 hr with vehicle (–), 100 nM/L E2, or 1 µM/L atrazine (Atr). (A) mRNA expression of ERα was evaluated by semiquantitative RT-PCR; the values of housekeeping gene 36B4 were determined as a control. (B) Immunoblot of ERα from BG-1 cells, with 100 nmol β-actin serving as a loading control. Results in (A) and (B) are representative of three independent experiments. (C) ERα binding assay using increasing concentrations of atrazine.

**Proliferation assay.** For the quantitative proliferation assay, we seeded 10,000 cells in 24-well plates in regular growth medium. Cells were washed once they had attached and then incubated in medium containing 2.5% CS-FBS with the indicated treatments. Medium was renewed every 2 days (with treatments), and cells were trypsinized and counted in a hemocytometer on day 6. The day before treatments, 200 ng/L of the indicated short hairpin RNA was transfected using FuGENE 6 Reagent as recommended by the manufacturer, and then renewed every 2 days before counting.

**Statistical analysis.** Statistical analysis was performed using analysis of variance followed by Newman-Keuls testing to determine differences in means. *p-Values < 0.05 are considered statistically significant.

**Results**

Atrazine does not activate ERα in cancer cells. Based on the evidence that atrazine produces early onset and increased incidence of estrogen-sensitive tumors in different experimental models (Cooper et al. 2007), we first evaluated whether atrazine could activate a transiently transfected ER reporter gene in estrogen-sensitive ovarian (BG-1), breast (MCF-7), and endometrial (Ishikawa) cancer cells. Exposure to 100 nM E2 induced a strong ERα transactivation that was absent in the presence of 10 µM of the ER antagonist ICI in all cell contexts evaluated (Figure 2A–C). In contrast, treatments with 1 µM atrazine and even concentrations ranging from 1 nM to 10 µM (data not shown) failed to stimulate luciferase expression or to block that observed upon addition of E2 (Figure 2A–C). Moreover, atrazine did not activate an expression vector encoding ERβ transiently transfected in ERβ-negative SkBr3 breast cancer cells (Figure 2D).

To confirm that atrazine is not an ERα agonist and to examine whether ERβ could respond to atrazine, we turned to a completely heterologous system. Chimeric proteins consisting of the DNA binding domain of the yeast transcription factor Gal4 and the ERα or ERβ HBD transiently transfected in SkBr3...
Atrazine neither regulates ERα expression nor competes with estrogen binding to ERα. Considering that the down-regulation of ERα induced by an agonist has been considered an additional hallmark of receptor activation (Santagati et al. 1997), we further investigated whether atrazine could modulate ERα expression in BG-1 cells, which lack ERβ (data not shown), and express a receptor expression pattern similar to that found in primary ovarian tumors (Bardin et al. 2004; Geisinger et al. 1989). As shown in Figure 3A,B, 100 nM E2 down-regulated ERα at both mRNA and protein levels, whereas 1 µM atrazine did not produce any modulatory effect. In agreement with these results and those obtained in transfection experiments, atrazine showed no binding capacity for ERα (Figure 3C), as previously reported (Cooper et al. 2007). Altogether, our findings rule out that the estrogen action of atrazine occurs through binding and direct activation of ERα.

Aromatase activity is not induced by atrazine. Given that atrazine is able to up-regulate aromatase expression and function in different cell contexts (Cooper et al. 2007; Fan et al. 2007a, 2007b; Reberge et al. 2004; Sanderson et al. 2000, 2001), we then determined aromatase activity by tritiated water release assays in BG-1 cells. As shown in Figure 4, 1 µM atrazine did not stimulate aromatase activity, which in contrast was strongly induced in human H295R adrenocorticotocarcinoma cells previously used as a model system to assess aromatase catalytic activity (Heneweier et al. 2004; Sanderson et al. 2001). In addition, the low aromatase protein expression detected in BG-1 cells did not increase upon exposure to 1 µM atrazine (data not shown). Hence, atrazine is neither an ERα activator nor an aromatase regulator in estrogen-sensitive ovarian cancer cells.

ERK phosphorylation is stimulated by atrazine. In recent years, numerous reports have demonstrated that estrogens and xenobiotics can generate rapid signaling via second messenger systems such as Ca2+, cAMP, nitric oxide, and G-proteins, which in turn leads to activation of different downstream kinases (Bulayeva and Watson 2004; Watson et al. 2007).

To evaluate whether the potential estrogenic activity of atrazine is exerted through a rapid cellular response, we investigated its ability to produce ERK phosphorylation in BG-1 cells. Interestingly, atrazine stimulated ERK phosphorylation, although a higher concentration and prolonged time period were required to trigger this biochemical response compared with E2 (Figures 5A,B, 6A). ERK activation was also delayed in the presence of 1 µM atrazine compared with 100 nM E2 in 2008 ovarian cancer cells (Figure 6D), which present a receptor expression similar to that of BG-1 cells (Safei et al. 2005). To determine the transduction pathways involved in ERK activation by atrazine, cells were exposed to 100 nM E2 and 1 µM atrazine along with specific inhibitors widely used to pinpoint the mechanisms contributing to ERK phosphorylation (Bulayeva and Watson 2004). Of note, the ER antagonist ICI, the EGFR inhibitor AG and the ERK inhibitor PD prevented ERK activation induced by both E2 and atrazine, whereas GFX, H89, and WM, inhibitors of protein kinase C (PKC), protein kinase A (PKA), and phosphoinositide 3-kinase (PI3K), respectively, did not (Figure 6B,C,E,F). Considering that in a previous study ICI was able to trigger ERK phosphorylation (Filardo et al. 2000), we exposed SKBr3 cells to increasing concentrations of ICI. We observed no ERK activation after...
regulation of c-fos observed in BG-1 and 2008 response (Figure 8). Interestingly, the up-and the transduction pathways involved in this are also regulated by atrazine in a rapid manner.

Hence, atrazine is able to stimulate the expression of diverse estrogen target genes without an inhibitor activity. Thus, in ovarian cancer cells, atrazine involves ERα and the EGFR/MAPK pathway to trigger c-fos protein increase.

Transduction pathways involved by atrazine in the up-regulation of c-fos protein levels. Using c-fos expression as a molecular sensor of atrazine action at the genomic level, we sought to determine whether c-fos protein levels are also regulated by atrazine in a rapid manner and the transduction pathways involved in this response (Figure 8). Interestingly, the up-regulation of c-fos observed in BG-1 and 2008 cells after a short treatment (2 hr) was abolished by the ER antagonist ICI, the EGFR inhibitor AG, or the ERK inhibitor PD (Figure 8). On the contrary, GFX, H89, and W/M, inhibitors of PKC, PKA, and PI3K, respectively, did not interfere with c-fos stimulation (Figure 8). Thus, in ovarian cancer cells, atrazine involves ERα and the EGFR/MAPK pathway to trigger c-fos protein increase.

Table 1. mRNA expression (mean percent variation ± SD) induced by 100 nM E2 and 1 μM atrazine in BG-1 cells.

| Gene          | E2       | Atrazine       |
|---------------|----------|----------------|
|               | 1 hr     | 24 hr          | 1 hr  | 24 hr |
| c-fos         | 422 ± 28*| 229 ± 17*      | 269 ± 21* | 120 ± 9 |
| PR            | 228 ± 18*| 229 ± 18*      | 122 ± 18 | 180 ± 11* |
| pS2           | 175 ± 17*| 270 ± 21*      | 99 ± 19 | 187 ± 20* |
| Cathepsin D   | 106 ± 9  | 217 ± 16*      | 102 ± 5 | 109 ± 6  |
| Cyclin A      | 262 ± 22*| 293 ± 23*      | 220 ± 20* | 190 ± 22* |
| Cyclin D1     | 258 ± 19*| 242 ± 19*      | 107 ± 4 | 118 ± 8  |
| Cyclin E      | 120 ± 11 | 343 ± 21*      | 118 ± 8 | 119 ± 10 |

The values calculated by optical density in cells treated with vehicle were set at 100%, and the expression induced by treatments is presented as percent variation.

* p < 0.05 compared with vehicle.

Figure 7. ERK1/2 phosphorylation (pERK1/2) in SKBr3 cells treated for 20 min with vehicle (–) or increasing concentrations of ICI.

Figure 8. Immunoblots of c-fos from BG-1 (A,B) and 2008 (C,D) cells treated for 2 hr with vehicle (–), 100 nM/L E2, or 1 μM/L atrazine (Atr) in combination with 10 μM/L ICI, AG, PD, GFX, H89, or WM, inhibitors of ER, EGFR, MEK, PKC, PKA, and PI3K, respectively. β-Actin served as a loading control.

Figure 9. Immunoblots of c-fos from BG-1 (A,B) and 2008 (C,D) cells after silencing ERα and GPR30 expression. Cells were transfected with control siRNA or siRNA-ERα (A,C) or with vector or shGPR30 (B,D) and treated for 2 hr with vehicle (–) or 100 nM/L E2 or 1 μM/L atrazine (Atr). Efficacy of ERα and GPR30 silencing was ascertained by immunoblots, as shown in side panels. β-Actin served as a loading control.
expression after silencing either ERα or GPR30 in BG-1 and 2008 cells (Figure 9). To evaluate whether atrazine could induce a rapid response in a cell context expressing GPR30 alone, we turned to ER-negative SkBr3 breast cancer cells. Notably, both ERK phosphorylation and c-fos induction stimulated by atrazine were abolished after silencing GPR30 (Figure 10), indicating that the response to atrazine is differentially regulated according to cancer cell type.

The proliferation of ovarian cancer cells induced by atrazine occurs through GPR30 and requires both ERα and EGFR/MAPK-mediated signaling. The aforementioned results were recapitulated in a more complex physiologic assay such as cell growth. We observed that both E2 and atrazine induced the proliferation of BG-1 and 2008 cells in a concentration-dependent manner (Figure 11A,E). Moreover, the growth effects elicited by E2 and atrazine were no longer evident in the presence of AG and PD (Figure 11B,F) or after silencing the expression of either GPR30 or ERα (Figure 11C,D,G,H), indicating that both receptors, along with the EGFR/MAPK transduction pathway, are involved in the growth effects as well as in the c-fos expression profile described above.

Discussion

In the present study, we demonstrated for the first time that atrazine exerts an estrogen-like activity in ovarian and breast cancer cells through GPR30, which is recently of interest because of its ability to mediate rapid estrogen activity in ovarian and breast cancer cells irrespective of ERα expression and despite a low binding affinity for GPR30 etopically expressed in HEK-293 cells (Thomas and Dong 2006). In line with these findings, an efficient competitor of E2 for endogenous GPR30 in SkBr3 cells, such as an ortho, para-dichlorophenyl dichloro-ethene (DDE) derivative, was ineffective in binding to recombinant GPR30 (Thomas et al. 2005; Thomas and Dong 2006). Likely, the interaction of atrazine with GPR30 is facilitated by the relative abundance of this membrane receptor in cancer cells with respect to cells engineered to express recombinant GPR30, and/or yet unknown factors may contribute to the binding to GPR30 by these contaminants.

Regarding the role of ERα, we proved that a complex interplay between GPR30 exists, as previously reported with some growth factor receptors (Migliaccio et al. 2006), but the presence of GPR30 along, as demonstrated in SkBr3 breast cancer cells. Although E2 exhibited an exclusive up-regulation of target genes through direct activation of ERα, the GPR30–EGFR transduction pathway was involved in estrogen-induced proliferation of ovarian tumor cells, as evidenced by silencing GPR30 and using specific pharmacologic inhibitors.

A variety of environmental contaminants exhibit binding affinities for GPR30 and agonist activities similar to those for ERs (Thomas and Dong 2006). In the present study atrazine triggered rapid biological responses through GPR30 in both ovarian and breast cancer cells irrespective of ERα expression and despite a low binding affinity for GPR30 etopically expressed in HEK-293 cells (Thomas and Dong 2006). In line with these contaminants, an efficient competitor of E2 for endogenous GPR30 in SkBr3 cells, such as an ortho, para-dichlorophenyl dichloro-ethene (DDE) derivative, was ineffective in binding to recombinant GPR30 (Thomas et al. 2005; Thomas and Dong 2006). Likely, the interaction of atrazine with GPR30 is facilitated by the relative abundance of this membrane receptor in cancer cells with respect to cells engineered to express recombinant GPR30, and/or yet unknown factors may contribute to the binding to GPR30 by these contaminants.

Figure 11. Proliferation of BG-1 (A–D) and 2008 (E–H) cells exposed to E2 or atrazine (Atr). (A,D) Proliferation of cells in response to increasing concentrations of E2 or Atr. (B–H) Proliferation of cells treated with vehicle (–), 100 nmol/L E2, or 1 μmol/L Atr with or without 10 nmol/L AG or PD (B,F) or transfect with vector or shGPR30 (C,G) or with control siRNA or siRNA-ERα (D,H). See “Materials and Methods” for details of experiments. Proliferation of cells receiving vehicle was set as 100%, and the cell growth induced by treatments was calculated. Values shown are mean ± SD of three independent experiments performed in triplicate. Efficacy of ERs and GPR30 silencing was ascertained by immunoblots (Figure 5). *p < 0.05 compared with treated cells.

Figure 10. ERK1/2 phosphorylation (A) and c-fos expression (B) after silencing GPR30 in SkBr3 cells treated with vehicle (–) or 1 μmol/L atrazine (Atr). (C) The efficacy of GPR30 silencing was ascertained by immunoblots. β-Actin served as a loading control.
molecular mechanisms involved remain to be elucidated. Our study and previous investigations indicate that environmental estrogens exert pleotropic actions by directly binding to ERα as well as through GPR30–EGFR signaling, which can engage ERα depending on the receptor expression pattern present in different cell types. This mode of action of xenoestrogens fits well with the results obtained after silencing GPR30 or ERα expression in ovarian cancer cells, because silencing each gene prevented the growth response to atrazine.

Our data recall the results of previous studies showing that xenoestrogens mimic rapid estrogen action in several animal and cell models (Bulayeva and Watson 2004; Loomis and Thomas 2000; Nadal et al. 2000; Ruehlmann et al. 1988; Watson et al. 1999, 2007). Particularly, in GH3/B6 or F10 pituitary tumor cells, diverse xenoestrogens induced ER phosphorylation with a temporarily distinct activation pattern compared with E2 (Bulayeva and Watson 2004). In the latter study, on the basis of the inhibitory activity exerted by ICI, the authors hypothesized that an ER localized to the plasma membrane could mediate the ER phosphorylation response by xenoestrogens, depending on their different ER binding affinities. Moreover, the authors suggested that the signaling cascades leading to ER activation may involve the nature of membrane ERs and their ability to interact with various signaling partners (Bulayeva and Watson 2004). Interestingly, our findings have provided evidence that ERα may be involved by xenoestrogens without a direct binding activity and produce relevant responses such as ER phosphorylation, gene expression, and cell growth.

A subset of estrogen-sensitive cell tumors can proliferate independently from ER expression (i.e., ER-negative cells). In this condition, well represented by SkBr3 breast cancer cells, GPR30–EGFR signaling may still allow for environmental estrogen activity as we have shown in the present study as well as in a previous study (Maggiolini et al. 2004). Hence, multiple transduction pathways triggered simultaneously at the membrane level, as well as within each cell type, may contribute to the nature and magnitude of biological responses to distinct estrogenic compounds. These consequently should be examined individually for their complex mechanistic and functional outcomes that result from interaction with a different repertoire of receptor proteins.

Atrazine, a potent endocrine disruptor, is the most common pesticide contaminant of groundwater and surface water. Here, we have provided novel insight regarding the potential role of GPR30 in mediating the action of atrazine in endocrine-related diseases, such as estrogen-sensitive tumors.

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