Molecular Pathways: Current Role and Future Directions of the Retinoic Acid Pathway In Cancer Prevention and Treatment

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Abstract

Retinoids and their naturally metabolized and synthetic products (e.g. all-trans retinoic acid, 13-cis retinoic acid, bexarotene) induce differentiation in various cell types. Retinoids exert their actions mainly through binding to the nuclear retinoic acid receptors (α, β, γ), which are transcriptional and homeostatic regulators whose functions are often compromised early in neoplastic transformation. The retinoids have been investigated extensively for their utility in cancer prevention and treatment. Success has been achieved with their use in the treatment of subtypes of leukemia harboring chromosomal translocations. Promising results have been observed in the breast cancer prevention setting, where fenretinide prevention trials have provided a strong rationale for further investigation in young women at high-risk for breast cancer. Ongoing phase 3 randomized trials investigating retinoids in combination with chemotherapy in non-small cell lung cancer aim to definitively characterize the role of retinoids in this tumor type. The limited treatment success observed to date in the prevention and treatment of solid tumors may relate to the frequent epigenetic silencing of RARβ. Robust evaluation of RARβ and downstream genes may permit optimized use of retinoids in the solid tumor arena.
Background

Vitamin A is derived from animal and plant food sources and has critical functions in many aspects of human biology. Its natural derivatives and metabolized products (retinoids) such as β-carotene, retinol, retinal, isotretinoin, all-trans retinoic acid (ATRA), 9-cis retinoic acid, and 13-cis retinoic acid have important roles in cell differentiation, growth, and apoptosis (1). Synthetic retinoids are also available and include bexarotene and fenretinide. In clinical practice, retinoids have a wide range of dermatologic indications including for psoriasis, acneiform and keratinization disorders (2). Systemic retinoids are approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T cell lymphoma (3) and acute promyelocytic leukemia (APL) (4, 5). However, the chemopreventive and therapeutic effects of retinoids in solid tumors remain controversial. Therefore, an overview of the research to date and future directions in this area is the focus of this review.

Retinoic Acid and the Retinoic Acid Receptor Pathway

Retinoic acids (RAs) exert their functions through their specific receptors. There are two distinct classes of receptors including retinoic acid receptors (RARs) and retinoic X receptors (RXRs). Each class contains three different subtypes—α, β, and γ (6). ATRA and fenretinide can bind specifically to RAR, 13-cis RA and bexarotene only to RXR, and 9-cis RA binding to RAR or RXR (1, 5) (Table 1). The expression of these receptors is regulated either by the receptors themselves, other nuclear receptors such as ERα, or by other subtypes in the same family (5, 7). Upon the binding of ligands, RARs and RXRs form heterodimers and function as ligand-dependent transcription factors to activate their downstream effectors by binding to the retinoic acid response elements (RAREs) located in the 5'-region of RA downstream genes (5). The above model of RARs or RXRs function via binding to RARE is considered the RA classical or genomic pathway. Activation of the classical pathway will trigger cell differentiation, cell arrest, and eventual apoptosis (8).

The function of retinoic acid and its receptors involves not only the classical pathway, but also multiple other important pathways. RAs have been shown to regulate NF-κB (9), INF-gamma (10), TGFβ (11), VEGF (12), MAPK (13), and chromatin remodeling (14). Furthermore, RARs and RXRs can form heterodimers with other types of receptors including the estrogen receptor-α (ERα) (7, 15), AP-1 receptor(16), peroxisome proliferator-activated receptor (PPARs) (17), liver X receptors (LXRs) (18, 19), and vitamin D receptor (VDR) (20) (Figure 1). When RARs/RXRs heterodimerize with these receptors, they are involved in regulating their partner receptor’s pathways, referred to as non-classical or non-genomic pathways (5). Interestingly, these pathways often regulate processes that have functions opposite to the classical pathway. For example, a study has shown that RA activation of the PPARβ/δ pathway resulted in upregulation of prosurvival genes (17), contrary to the known differentiation function of RARs and RXRs in response to RA. The function of RAs which involves non-genomic pathways may provide opportunities for cancer cells to develop resistance to RA treatment, discussed later in this review. Another important function of retinoic acid is the regulation of stem cell differentiation (11). Retinoic acids target stem cells via both genomic and non-genomic pathways such as the Notch pathway and inflammation (10, 11). In summary, RAs and their receptors play important roles as regulators of critical processes in cells.
Retinoids and Cancer

The retinoids have been investigated extensively for the prevention and treatment of cancer, predominantly because of their ability to induce cellular differentiation and arrest proliferation. RA-regulated tumor suppressor genes, when expressed, can inhibit tumor growth (21). Among the three RARs, RARβ has been well known for its tumor suppressive effects in epithelial cells (5, 8, 22). Exogenous expression of the RARβ gene can cause RA-dependent and RA-independent apoptosis and growth arrest (23). RARβ induced growth arrest and apoptosis is mediated through RARα (24). As RA ligand-bound RARα binds to the retinoic acid response element (RARE) on the RARβ promoter, multiple activator proteins assemble at the site, and result in the upregulation of the RARβ gene (5). The expression of RARβ results in the transactivation and expression of a number of its target genes that mediate cell differentiation and death (5, 6, 8). The ability of ATRA to initiate differentiation of promyelocytic leukemic cells to granulocytes is the basis of the dramatic success of retinoic acid therapy of acute promyelocytic leukemia harboring the RAR/PML translocation (4) and confirms the important role of RARβ in tumor growth inhibition. It is also becoming increasingly clear that RARβ expression is lost early in carcinogenesis or is epigenetically silenced (25) in many solid tumors, providing an opportunity for novel treatment strategies to be investigated using retinoids together with epigenetic modifiers that promote re-expression of silenced genes, described further below.

Clinical-Translational Advances

The retinoids have an established role in the treatment of certain hematological malignancies, with FDA approval for use in cutaneous T cell lymphoma and APL. Bexarotene (an RXR-selective retinoid or rexinoid) is associated with an overall response rate of approximately 50% in patients with refractory advanced stage mycosis fungoides, a cutaneous T cell lymphoma (3). ATRA, a synthetic retinoid, exhibited improvements in disease-free and overall survival when compared with chemotherapy alone in APL, with long-term remissions occurring in almost 70% of cases (4). The success of retinoids in treating this disease relates to the underlying chromosomal translocation and production of the PML/RARα fusion protein and the ability of retinoids to induce differentiation and inhibition of cell growth in this setting (26, 27). Clinical trials investigating the role of retinoids in the prevention and treatment of solid tumors will now be outlined with a focus on cancers of the upper aerodigestive tract (oropharyngeal and lung) and breast. (Table 1)

Head and Neck Cancer

Premalignant oropharyngeal lesions have been shown to express low levels of RARβ, and it has been hypothesized that restoration of expression could reinstate normal growth and differentiation patterns. Indeed, RARβ messenger ribonucleic acid (mRNA) expression was induced with retinoid therapy in specimens of oral mucosa available pre and post 13-cis RA (n=39). The levels of RARβ mRNA increased in the specimens from 18 of the 22 patients who had responses to 13-cis RA and in 8 of the 17 specimens from the patients without responses (p = 0.04) suggesting RARβ mRNA as a biomarker of response to therapy (28). An early randomized trial compared the use of 13-cis RA to placebo in patients with premalignant oral leukoplakia, with a dramatic decrease in the size of the lesions observed in 67% and 10% of patients respectively. Unfortunately, relapse occurred in the majority of patients within a few
months (29). In a follow up trial, a lower dose of 13-cis RA was significantly more active against leukoplakia than beta carotene and was well tolerated (30). A Cochrane review has subsequently concluded, however, that there is not sufficient evidence currently to support the use of any agent to prevent the progression of oral leukoplakia to oropharyngeal cancer (31). The retinoids have also been evaluated in patients with a diagnosis of localized head and neck cancer after completion of surgery or radiation therapy with little promise overall (32). Since evidence supporting RA’s non-genomic action such as through inhibiting jun N-terminal kinase (JNK) phosphorylation or inhibiting the transactivation potential of NF-κB has been reported in head and neck cancer, this mode of action could have contributed to its limited success in treatment of this type of cancer (9, 33, 34)

**Lung Cancer**

A number of studies have also investigated the role of single agent retinoids in the prevention of lung cancer; in those at high risk of lung cancer (primary chemoprevention) (35-39), those with existing pre-malignant changes in bronchial epithelium or sputum (secondary chemoprevention) (40-42) and in those with a history of lung cancer (tertiary chemoprevention) (43-45). These studies have not indicated a benefit with use of retinoids in these settings, and indeed an increased risk of lung cancer was observed in smokers in some studies (36, 38). Interestingly, one study was designed to investigate whether either of two retinoid-based regimens could reverse RARβ expression loss in former smokers. A statistically significant restoration of RARβ expression and reduction of metaplasia were found in the 9-cis-RA group when compared to placebo (46). A recent study found a dual growth promoting and repressive role for RARβ2 in lung cancer cells, which may help explain the inconsistent results observed in clinical trials (47).

Based on preclinical observations of the ability of retinoids to enhance chemotherapy-induced cytotoxicity (48, 49), clinical studies have combined retinoids with chemotherapy in the treatment of lung cancer. A randomized phase 2 study of paclitaxel and cisplatin with or without ATRA was performed in patients with advanced non-small cell lung cancer (NSCLC, n=107) (50). Both response rate (55.8% versus 25.4%) and median progression-free survival (8.9 versus 6 months) favored the arm incorporating ATRA. An association between RAR-β2 expression and response rate was investigated, but no significant association was identified, perhaps due to the small numbers of tumor samples that expressed the gene (10%, n=6) (50). Based on the promising clinical results, a phase 3 trial is now in the planning stages and aims to evaluate the benefit of RAR-β2 and RARα expression as a response biomarker.

In contrast to these results, a phase 3 trial of bexarotene in combination with chemotherapy yielded disappointing results despite promising single agent and phase 2 data (51). Cisplatin and vinorelbine with or without bexarotene were administered to 623 patients with chemotherapy-naive advanced NSCLC. There was no difference in survival (the primary study endpoint) between the arms (52).

**Breast Cancer**

Fenretinide has been extensively studied in breast cancer prevention trials. Supportive preclinical studies revealed its inhibition of mammary carcinogenesis in animal models (53), and
the selective accumulation of fenretinide in human breast tissue has been documented (54). The role of fenretinide in reducing contralateral or second ipsilateral breast cancer in patients with early breast cancer (n=2867) revealed no significant difference in these endpoints at 8 year follow up (55). However, an unplanned exploratory analysis indicated a 35% reduction in events in premenopausal women, with a trend towards a detrimental effect being observed in postmenopausal women. These results have prompted a phase 3 primary prevention trial in premenopausal women at high risk of breast cancer (56).

Efforts to improve on these results have also included a biomarker trial of fenretinide and low-dose tamoxifen in premenopausal women at high risk of breast cancer. Tamoxifenn is an approved agent for breast cancer prevention in high risk individuals. Despite promising preclinical data supporting the combination (57) and its favorable effects on plasma IGF-I levels and mammographic density in this clinical trial, the combination of low-dose tamoxifen plus fenretinide did not reduce breast cancer events compared to placebo. A numerical reduction in the annual odds of breast cancer was observed with both single agent tamoxifen and fenretinide, supporting ongoing investigation of fenretinide in the breast cancer prevention setting (58).

Clinical trials investigating the retinoids as a single agent in metastatic breast cancer have been disappointing. In a phase 2 trial investigating single agent 13-cis RA in metastatic breast cancer that was refractory to treatment, no objective responses were observed (59). ATRA administration as a single agent yielded a clinical benefit rate of 26.8% (60). A phase 2 trial of oral bexarotene (n=148) in metastatic breast cancer reported a clinical benefit rate of approximately 20% with minimal toxicity observed (61).

Binding of RARs throughout the genome is highly coincident with ERα binding in an ER-dependent manner at ER-binding sites, potentially by maintaining ER-cofactor interactions. These findings suggest that RARs, acting in a nongenomic manner, can cooperate with ERα for effective transcriptional activity in breast cancer cells (7, 15). Based on the known interaction between the RARs and the ERα pathways, a clinical trial was performed in patients with hormone-responsive metastatic breast cancer which investigated the addition of hormonal therapy to retinoids. No benefit to the combination therapy was observed at 8 year follow up (62). Finally, a phase 2 single arm trial of ATRA plus paclitaxel was performed in patients with metastatic breast cancer (n=17). Partial response was observed in 3 patients (17.6%) and stable disease in ten patients (58.8%), with a clinical benefit rate of 76.4%. Although these results appear promising, they are comparable with historical reports with paclitaxel alone (63).

**Potential Mechanisms of Resistance**

Although pharmacological doses of retinoids have proven effective in the treatment of hematologic malignancies (64), clinical trials in the prevention and treatment setting in a number of solid tumors including lung cancer and breast cancer have failed to show significant benefit to date (51, 63). The lack of a robust biomarker of response to therapy is one reason for this failure. In addition, there are a number of potential mechanisms of resistance to these therapies that have been proposed.
Epigenetics

In solid tumors, RARβ gene expression is frequently lost in primary tumors and their metastasis compared to adjacent non-cancerous tissues (65, 66). This provides a possible explanation as to why treatment using RAs in solid tumors such as breast cancer have previously failed. Our laboratory and others have provided extensive evidence that RARβ is silenced in breast cancer by epigenetic modification including both methylation at the promoter region of the gene and a compacted chromatin structure (25, 67). Epigenetically silenced RARβ has been shown to be re-expressed in the presence of DNA methyltransferase inhibitors (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors in RARβ2 silent breast cancer cells (67). Treatment with an HDAC inhibitor combined with 9-cis RA resulted in regression of prostate and breast cancer xenografts (67, 68). It is possible that the addition of epigenetic modifiers to RA-based therapy will be needed to reactivate RARβ in RARβ-silent tumors in order to accomplish significant growth inhibition (Figure 2). With this in mind, a number of clinical trials have incorporated epigenetic modifiers with retinoids in an attempt to improve the outcomes observed with single agent retinoids, and thereby potentially overcome resistance. Entinostat (MS-275), a HDAC inhibitor, has been combined with 13-cis RA in a phase 1 trial in patients with advanced solid tumors and lymphomas. The combination was reasonably well tolerated and a recommended phase 2 dose was identified for future studies (69). A single arm phase 2 study has also been reported which investigated the efficacy of 5-azacitidine (DNMT inhibitor), valproic acid (HDAC inhibitor) and ATRA in patients with hematologic malignancies (70). Best responses to this combination of agents included 14 complete responses and 3 partial responses to therapy. It is important to note that in the clinical trials described to date, the RARβ status of the tumors was not assessed prior to therapy. Tumors that do not express the RARβ receptor are unlikely to respond to RA treatment. The efficacy of retinoids may be further enhanced with the addition of cytotoxic agents to the combination of retinoids and HDAC inhibitors, perhaps by debilitating several critical interacting pathways that the cancer cell depends on for continued growth and proliferation. We have demonstrated that the combination of the HDAC inhibitor entinostat, ATRA, and low dose chemotherapy yielded the greatest inhibition of tumor cell growth in vitro and in human tumor breast cancer xenografts (71).

Cancer stem cells

Another potential mechanism of resistance to retinoids in solid malignancies is the presence of cancer stem cells. Many studies have attempted to target cancer stem cells with differentiation treatments including RAs (72, 73). Our laboratory has found that treatment of tumors using a HDAC inhibitor, ATRA, and low dose doxorubicin not only results in striking tumor regression, but also significantly reduces the number of cancer stem cells (unpublished data). Therefore, HDAC treatment may induce differentiation in the stem-like tumor cells, which may circumvent resistance to standard chemotherapy or ATRA treatment alone. Further studies are needed to further delineate the role of RA in targeting these cells that are generally deemed treatment resistant.

Other potential mechanisms of resistance

Cancer cells may silence or repress RARβ by mechanisms other than epigenetic modulation in order to initiate and promote their growth and resist treatment with RA. A number
of alternative mechanisms have been proposed including the loss of coactivators (74), increased RA metabolism (75), decreased RA availability (76), and impaired RARα signaling (77). For example, studies have showed that AF2 co-activators of the RAR-Thyroid Hormone Receptor complex are often lost in human lung cancer (74). The loss of AF-2 cofactors results in low levels of transcribed RARβ, suggesting an important function of these cofactors in mediating RARβ expression. Another study demonstrated that impaired RARα function failed to facilitate changes in RARβ’s chromatin structure necessary for RARβ activation, implicating a critical role for RARα in controlling RARβ expression.

Potential mechanisms of resistance that are independent of RARβ have also been suggested. Aberrant p53 expression, for example, has been associated with 13-cis RA resistance in the clinic (78); RA, it appears, can promote intrinsic transactivation of p53 (79). It is also possible that cross talk between the RAR and ER in breast cancer can create opportunities for cancer cells to bypass pathways inhibited by targeted therapies such as RA or hormonal therapies (7, 19).

Conclusions and Future Directions

In summary, retinoids have been investigated extensively for their utility in solid tumor cancer prevention and treatment. Promising results have been observed in the breast cancer prevention setting, where fenretinide prevention trials have provided a strong rationale for a new trial in young women at high-risk for breast cancer. Clinically relevant outcomes have also been observed with the use of retinoids combined with chemotherapy in NSCLC, prompting the development of confirmatory phase 3 randomized trials. Further delineation of the mechanisms of action and resistance of retinoids in solid tumors may provide the rationale for future studies and result in clinical benefit for patients. Ongoing and future studies which combine retinoids with epigenetic modifiers such as the HDAC inhibitors, as well as standard cytotoxic agents, tyrosine kinase inhibitors and other novel agents are more likely to yield clinically relevant outcomes than observed with single agent therapy. Novel RA metabolism blocking agents (RAMBAs) are also undergoing investigation at this time (80). Finally, clinical trialists should be encouraged to incorporate correlative endpoints in their studies in order to identify accurate biomarkers of response to retinoid therapy.

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Author Contributions

All three authors contributed equally to the manuscript.

Competing Financial Interests

None

The authors declare no conflicts of interest.
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**Figure Legends:**

**Figure 1:** The Retinoic Acid Receptors and their action

In a series of enzymatic steps, Vitamin A (retinol) is metabolized through the oxidizing action of retinaldehyde (RDH) to retinal, and by retinaldehyde dehydrogenase (RALDH) to retinoic acid (RA). RA has three different isomers including all-trans, 9-cis, and 13-cis-retinoic acids. Retinoic acid is transported to the nucleus by the protein Cellular Retinoic Acid Binding Protein (CRABP) and delivered to the retinoic acid receptor α (RARα). RARα heterodimerizes with, and binds to RA responsive elements (RARE) present most often in gene promoters. In the classical pathway of RA action, RA binds to dimers of RARα and rexinoid receptors (RXR α, β, or γ) to induce expression of its downstream target genes, including RARβ. Upon activation, RARβ can regulate its own expression and that of its downstream genes, which function are mainly to inhibit cell growth. Alternatively, RA can be bound and transported to the nucleus by other factors such as FABP5. This delivers RA to other non-classical receptors such as PPARβ/δ and ERα which activate non-genomic pathways such as PDK-1/Akt or the ERα pathway. Contrary to the differentiation functions attributed to the classical pathway, the non-genomic pathways exert strong anti-apoptotic and proliferative effects on cancer cells. It is believed that the classical and non-genomic pathways are controlled by the relative abundance of their own ligands. RA has a stronger affinity for RARs compared to the other receptors and the classical pathway plays a dominant role over the non-genomic pathways. Thus, if RA is present with other ligands such as estrogen, signaling through the classical pathway is preferred to result in cell differentiation and growth inhibition.

**Figure 2.** Mechanism of activation of RARβ, an important downstream effector of the RA pathway, in cancer growth inhibition.

Under conditions where RARα is functional and the RARβ promoter is not epigenetically silenced, physiological levels of RA can activate RARβ expression. A small number of solid tumors display this phenotype. Under less ideal conditions in which the RARβ promoter is hypoacetylated, pharmacological doses of RA are needed to activate RARβ. In the majority of solid tumor types, the RARβ promoter is methylated and/or the histones are significantly deacetylated. In this case, treatment with pharmacological doses of RA is not sufficient to overcome the repressive effect of epigenetic silencing. Epigenetic-modifying drugs such as DNA methytransferases or histone deacetylase (HDAC) inhibitors are needed to release the epigenetic stress and activate the RARβ gene. NCOR: nuclear receptor co-repressor 1; SMRT silencing mediator for retinoid and thyroid receptors
### Table 1. Select clinical trials evaluating retinoids in solid tumors

<table>
<thead>
<tr>
<th>Retinoid</th>
<th>Other names</th>
<th>Target</th>
<th>Clinical Trial Setting</th>
<th>Dose and Schedule</th>
<th>Study Outcome</th>
<th>Biomarker Evaluation</th>
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<tbody>
<tr>
<td>ATRA</td>
<td>Tretinoin, Retinoic Acid</td>
<td>Advanced NSCLC</td>
<td>Phase 2 randomized</td>
<td>20mg/m2/day commencing 1 week pre paclitaxel/cisplatin every 3 weeks (Arrieta JCO 2010)</td>
<td>RR (55.8% vs. 25.4%) and median PFS (8.9 vs. 6 months) favored the ATRA arm. Phase 3 study pending.</td>
<td>No significant association between RAR-β2 expression and response rate detected (n=60).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metastatic breast cancer</td>
<td>Phase 2 single arm</td>
<td>45 mg/m2/day for 4 days commencing 2 days pre weekly paclitaxel. (Arrieta JCO 2010)</td>
<td>RR (55.8% vs. 25.4%) and median PFS (8.9 vs. 6 months) favored the ATRA arm. Phase 3 study pending.</td>
<td>Clincial benefit rate of 76.4%. Note the majority of patients had not received prior paclitaxel.</td>
</tr>
<tr>
<td>13-cis RA</td>
<td>Isotretinoin, Accutane</td>
<td>Primary Prevention H+N cancer.</td>
<td>Induction phase: high dose (1.5 mg/kg) for three months, Maintenance phase: low dose (0.5 mg/kg/day) vs. beta carotene (30 mg/day) for nine months (8416267)</td>
<td>Induction phase (n=66): RR 55%, Maintenance phase (n=53): response or stable disease 92% (n=22 isotretinoin) vs. 45% (n=13 beta carotene)</td>
<td>Antitumor activity observed and recommended phase 2 dose determined.</td>
<td>No significant difference in RR or overall survival between the 3 arms.</td>
</tr>
<tr>
<td>9-cis RA</td>
<td>Alitretinoin, Roaccutane</td>
<td>Metastatic breast cancer</td>
<td>Induction phase: high dose (1.5 mg/kg) for three months, Maintenance phase: low dose (0.5 mg/kg/day) vs. beta carotene (30 mg/day) for nine months (8416267)</td>
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<td>Antitumor activity observed and recommended phase 2 dose determined.</td>
<td>No significant difference in RR or overall survival between the 3 arms.</td>
</tr>
<tr>
<td>Fenretinide</td>
<td>4-OH phenylretinamide</td>
<td>Primary prevention: women at high risk of breast cancer. Randomized double-blind 2x2 design. (n=235)</td>
<td>Tamoxifen 5 mg/day vs. Fenretinide 200 mg/day vs. the combination vs. placebo.</td>
<td>Low-dose tamoxifen plus fenretinide did not reduce breast cancer events vs. placebo. Numerical reduction in annual odds of breast cancer observed with both single agent tamoxifen and fenretinide.</td>
<td>Baseline IGF-I/mammographic density, as well as change in mammographic density did not predict breast cancer events.</td>
<td>No difference in rates of breast cancer in overall population, but 35% reduction in events in premenopausal women in unplanned exploratory analysis.</td>
</tr>
<tr>
<td>Bexarotene</td>
<td>RXR</td>
<td>Chemotherapy-naive advanced NSCLC. Phase 3 randomized. (n=623)</td>
<td>400mg/m2/day every 4 weeks with cisplatin/vinorelbine (Ramlau 2008, 18398154)</td>
<td>No survival benefit for addition of bexarotene to chemotherapy.</td>
<td>Grade 3-4 hypertriglyceridemia associated with longer median survival in an unplanned subgroup analysis.</td>
<td>No difference in rates of breast cancer in overall population, but 35% reduction in events in premenopausal women in unplanned exploratory analysis.</td>
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</table>

ATRA, all-trans retinoic acid; RAR, retinoic acid receptors; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression-free survival; RA, retinoic acid; RXR, retinoic X receptors; MS-275, entinostat; IM, intramuscular; H+N, head and neck; IGF, insulin-like growth factor.
Figure 1:

**Classical/genomic pathway**

With RA and w/o E2

- ERα
- E2
- ERE

- RARα

Induces differentiation
Inhibits growth

- RARE

- PPARβ/δ
- RXR
- PDK-1/Akt

**Non-genomic pathway**

With E2 and w/o RA

- RARα ERα
- E2

- PPARβ/δ
- RXR
- PDK-1/Akt

- c-MYC,
- Cyclin D1

Promotes cell growth

<table>
<thead>
<tr>
<th>Genetic pathway</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
</tr>
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<tbody>
<tr>
<td>Classical/genomic pathway</td>
<td>Retinoic acid (with RA and w/o E2)</td>
<td>E2, ERα, PPARβ/δ, RXR, PDK-1/Akt, c-MYC, Cyclin D1</td>
</tr>
<tr>
<td>Non-genomic pathway</td>
<td>RDH, RALDH, CRABP, FABP5</td>
<td>RARα, ERα, E2, PPARβ/δ, RXR, PDK-1/Akt, c-MYC, Cyclin D1</td>
</tr>
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</table>

**Key Proteins**

- ERα (Estrogen Receptor α)
- E2 (Estrogen)
- ERE (Estrogen Response Element)
- RARα (Retinoic Acid Receptor α)
- PPARβ/δ ( Peroxisome Proliferator-Activated Receptor β/δ)
- RXR (Retinoid X Receptor)
- PDK-1/Akt (Phosphatidylinositol-4,5-bisphosphate 3-kinase)
- c-MYC, Cyclin D1

**Pathway Details**

- **Retinol (Vitamin A)**: Converts to Retinal, which is then converted to Retinoic acid.
- **Retinoic acid** interacts with RARα, which activates or inhibits growth depending on the context.
- **ERα/E2 Interaction**: Activates or inhibits growth depending on the context.
- **PPARβ/δ/RXR Interaction**: Activates PDK-1/Akt, which is antiapoptotic.
- **c-MYC, Cyclin D1**: Promotes cell growth.

**Nucleus**

- **Cytoplasm to Nucleus**: diffusion of molecules

**Conclusion**

The diagram illustrates the interplay between retinoic acid, estrogen, and retinoid receptors in both genomic and non-genomic pathways, highlighting the complex regulatory mechanisms involved in cell growth and differentiation.
Figure 2:

- **RARα functional**
- **Unmethylated promoter**
- **Acetylated histones**

**Active RARβ at physiological levels of RA**

- **RARα functional**
- **Unmethylated promoter, OR**
- **Hypoacetylated histones**

**Inducible RARβ at pharmacological levels of RA**

- **RARα non-functional**
- **Methylated promoter, OR**
- **Hypoacetylated histones**
- **Binding of other mediators (PARP-1 or TopoIIβ)**

**Uninducible RARβ at pharmacological levels of RA. Other epigenetic modifiers are needed.**

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