Published in final edited form as:

Pharmacol Res. 2019 September ; 147: 104331. doi:10.1016/j.phrs.2019.104331.

The potential of retinoids for combination therapy of lung cancer: Updates and future directions

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Abstract

Lung cancer is the most common cancer-related death worldwide. Natural compounds have shown high biological and pharmaceutical relevance as anticancer agents. Retinoids are natural derivatives of vitamin A having many regulatory functions in the human body, including vision, cellular proliferation and differentiation, and activation of tumour suppressor genes. Retinoic acid (RA) is a highly active retinoid isoform with promising anti-lung cancer activity. The abnormal expression of retinoid receptors is associated with loss of anticancer activities and acquired resistance to RA in lung cancer. The preclinical promise has not translated to the general clinical utility of retinoids for lung cancer patients, especially those with a history of smoking. Newer retinoid nano-formulations and the combinatorial use of retinoids has been associated with lower toxicity and more favorably efficacy in both the preclinical and clinical settings. Here, we highlight epidemiological and clinical therapeutic studies involving retinoids and lung cancer. We also discuss the biological actions of retinoids in lung cancer, which include effects on cancer stem cell differentiation, angiogenesis, metastasis, and proliferative. We suggest that the use of retinoids in combination with conventional and targeted anticancer agents will broaden the utility of these potent anticancer compounds in the lung cancer clinic.

Keywords

Retinoids; Lung cancer; Retinoic acid receptor; Chemoresistance; Combination therapy

1. Introduction

Lung cancer represents the second most diagnosed cancer with a high incidence of mortality in both men and women worldwide [1]. Based on histology, there are two main types of lung

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Declaration of Competing Interest

The authors declare no conflict of interest regarding the publication of this review article.

cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Both types of lung cancer are epithelial in origin and have an inferior prognosis. A small subpopulation of cells may be responsible for the aggressive behavior and resistance to chemotherapy of these tumours [2]. This subpopulation of cells is known as tumour-inducing cells (TICs) or cancer stem cells (CSCs). Both TICs and CSCs have self-renewing, tumour-initiating, and multipotent differentiation properties. Compounds that target TICs and CSCs and promote their differentiation would be predicted to have considerable therapeutic potential in lung cancer patients in both the early and advanced settings [3].

Vitamins are essential for healthy growth, differentiation, and development of the human body. Like most vitamins, animals do not synthesize vitamin A, which must be supplemented in the diet mainly from plants sources. Retinoids are vitamers of vitamin A, derived from retinyl esters. Retinoids have several isoforms including β -carotene, retinol, retinal, all-*trans*-retinoic acid, and 13-*cis*-retinal, among others [4]. In mammals, retinoids regulate vision, cellular proliferation, differentiation, the immune system, growth of skin and bone tissue, and perhaps most importantly for cancer, the activation of tumour suppressor genes [5,6]. Retinoids inhibit the growth and progression of various malignancies, including cancers of the lung, mouth, prostate, and skin [7–10]. Retinoids also can modulate various signaling pathways and inhibit cell proliferation, growth factor expression, and cell cycle progression in many human cancers, including lung cancer [6]. Retinoids regulate cyclins, CDKs and cell cycle inhibitors to mainly arrest cells in the G1 phase [11]. Retinoids exert anticancer activities *via* binding to nuclear receptors, the retinoic acid receptors (RARs), RAR α , RAR β , and RAR γ and their isoforms (Table 1). The main inducible and aberrantly expressed retinoid receptor isoform in tumorigenesis is RAR β 2 [12]. Silencing of the RAR β 2 gene has been associated with retinoid resistance, and restoration of RAR β 2 expression can restore the anticancer activity of retinoids [13]. Epigenetic silencing of the RAR β 2 gene has also been documented in lung cancer [14,15]. Combinatorial use of retinoids with tyrosine kinase inhibitors (TKIs) can restore TKI sensitivity in TKI resistant lung cancer patients [16]. The retinoid derivative all-*trans* retinoic acid, also called retinoic acid (RA) is approved by the Food and Drug Administration (FDA) for the treatment of lymphoma and leukemia. RA inhibits many biological functions, such as tumour growth, angiogenesis, and metastasis [17,18]. RA treatment of human cancer cells and tumour xenografts suppresses cancer cell growth and promotes apoptosis and differentiation of CSCs [3].

Here, we summarize recent studies focused on molecular and cellular mechanisms regulated by retinoids in lung cancer. This review will also provide details on the clinical potential of retinoids in combination therapies for treatment naive and chemotherapy resistant lung cancer patients. We hope this review on retinoids and lung cancer provides useful insight and further understanding for researchers working in the field of lung cancer prevention and therapy.

2. Retinoids

Retinoids, a class of chemical compounds derivative from vitamin A, are implicated in stimulating cellular differentiation, growth, and development but also in inducing cell death

[29,30]. Retinoids are composed of a cyclic hydrophobic moiety connected to a polar moiety *via* a linker unit. Mammals cannot synthesize vitamin A which must be obtained in the diet mainly from plant pigments such as carotenes (β -carotene, β -cryptoxanthin, and α -carotene) or animal sources (cod liver oil, eggs, butter) [6]. After consumption, retinol can be stored in the form of retinyl esters in hepatic stellate cells. An irreversible oxidation reaction converts retinal into RA and other isoforms broadly categorized as retinoids [31]. Isoforms of retinoids are found in the all-*trans*; 13-*cis*; 9, 13-di-*cis*; 11-*cis*; 11, 13-di-*cis*; and 9-*cis* configurations. Biologically active endogenous retinoids are all-*trans* retinoic acid (ATRA); 11-*cis*-retinal; 3,4-didehydro-RA; 14-hydroxy-4, 14-retro-retinol; 4-oxo-RA; 4-oxo-retinol; and 9-*cis*-RA [6]. Based on molecular structure, retinoids are classified into first-, second-, and third-generations. First-generation comprises naturally occurring, nonaromatic retinoids obtain from retinyl palmitate, retinol, retinal, ATRA, 9-*cis*-RA, and 13-*cis*-RA. Second-generation consists of synthetic mono-aromatic retinoids such as etretinate and acitretin while the third-generation includes synthetic polyaromatic retinoids such as bexarotene and fenretinide. Chemical structures of common natural and synthetic retinoids are provided in Fig. 1.

3. Retinoids in lung cancer

Initially, retinoids were used to treat ophthalmological and dermatological related conditions in humans. However, in the last few decades, retinoids have received considerable attention as anti-carcinogens alone or in combination with existing therapies due to their CSCs differentiating properties. Epidemiological studies established an inverse relationship between vitamin A intake and cancer risk. Aberrant expression of retinoid receptors is also associated with malignancy in animal tissues and cancer cells. Retinoids have documented tumour suppressor activity in many animal cancer models, including tumours of the lung, breast, oral cavity and prostate. Furthermore, clinical studies supported the potential of retinoids to reverse pre-malignancy in human epithelial tissues and to promote the differentiation of CSCs, leading to growth inhibition of several cancers, including lung cancer. This review focuses on molecular and cellular mechanisms of action of retinoids in lung cancer treatment.

3.1. The anti-cancer effect of retinoids in lung cancer angiogenesis and metastasis

Metastasis refers to the spread of cancer cells from a primary site to a new location, thereby colonizing distant organs [32]. Cancer which has metastasized is responsible for most cancer-related mortality. Angiogenesis plays a crucial role in metastatic spread. Angiogenesis is the development of new blood vessels from pre-existing vasculature [33]. These blood vessels not only fulfil the metabolic requirement of a growing tumour but also provide a passage for the migration of cancer cells to distant sites. Malignant cells secrete multiple proangiogenic factors that trigger new blood vessel formation in nearby healthy tissues from extant cells. These factors include vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), hypoxia-inducible factors, platelet-derived growth factors (PDGF), matrix metalloproteinases (MMPs), and angiopoietin [34]. A high serum level of VEGF is an indicator of poor prognosis in lung cancer patients. Thus, VEGF has long been recognized as a potential target to inhibit tumour

progression and prolong survival. Sunitinib and sorafenib are small drug molecules which act by inhibiting multiple receptor tyrosine kinase (RTK) pathways involved in proangiogenic signaling [17]. Different classes of natural and synthetic retinoids have been found to display promising *in-vitro* and pre-clinical activities as anti-angiogenesis and angiopreventive agents.

RA appears to repress human colon adenocarcinoma cell movement by decreasing myosin light chain kinase (MLCK) expression and cancer cell metastasis [18]. Further investigation indicated that MLCK expression is upregulated in different types of tumours including lung, prostate, brain, liver, ovarian and melanoma [35–37]. Thus, preventing MLCK overexpression is a potential mechanism to oppose the metastasis of cancer cells.

Syndecan-1, a proteoglycan of lung epithelium is associated with tumour cell growth and invasiveness. RA positively regulates the syndecan-1 expression and inhibits invasion and metastasis in a mouse lung cancer model [38]. RA shows differential outcomes as an anti-angiogenic agent in various cancers. RA demonstrated anti-angiogenesis and anti-metastatic property in brain cancer and esophageal squamous cell carcinoma (ESCC). In glioma cells, RA shows anti-angiogenic activity by downregulating the expression of VEGF and HIF-1 α [39]. Similarly, RA suppressed the expression of angiopoietin 1 (AP1), angiopoietin 2 (AP2), and receptor Tie-2 leading to decreased angiogenesis in ESCC cells [40,41]. In contrast, RA supports survival and migration of lung tumour cells by ERK pathway activation [42] and inhibition of the ERK signaling pathway reverses this RA activity. Therefore, combinatorial treatment of RA with ERK inhibitors may serve to inhibit lung cancer migration and survival. RA showed minimal efficacy as a single agent in a phase II trial in metastatic NSCLC, while the combination with other chemotherapeutic agents has proved promising results [43]. Grace et al. showed that liposomal RA surpassed the anti-metastatic potential of free RA in a lung cancer mouse model [44]. Liposomal RA resulted in higher RA concentrations in lung tissue and restricted tumour nodule formation, thus providing a possible benefit in inhibiting lung metastasis.

Bexarotene, a synthetic retinoid, has demonstrated anti-angiogenic and anti-metastatic effects in lung cancer in a dose-dependent manner by activation of its dimerization partner PPAR γ [45]. *In-vitro* treatment of bexarotene decreased expression of VEGF, EGF and MMPs, and upregulated tissue inhibitors of matrix metalloproteinases (TIMPs) secretion in lung cancer cells. Furthermore, bexarotene inhibits angiogenesis by directly inhibiting human umbilical vein endothelial cell growth and indirectly inhibiting tumour cell-mediated migration of human umbilical vein endothelial cells. Fu et al. also demonstrated the inhibitory effect of bexarotene on tumour-induced angiogenesis [46]. Bexarotene negatively regulated the expression of VEGF and suppressed JNK and ERK activation. Despite these results and promising findings in a phase II trial, the addition of bexarotene to first-line chemotherapeutic drugs in a phase III trial did not increase the survival of patients with advanced NSCLC [47]. Studies also suggested that subcutaneous administration of low dose interleukin-2 with oral 13-*cis*-RA inhibited VEGF expression and enhanced progression-free and overall survival of advanced stage NSCLC patients [48]. The above studies suggest a potential therapeutic role for retinoids as a potent anti-metastatic and anti-angiogenic agent in suppressing lung cancer progression (Fig. 2).

3.2. Retinoids associated aberrant signaling in lung cancer

Aberrant signaling is a unique feature of cancer malignancy. In most cancers, at least one cell signaling pathway is deregulated. Deregulated signaling pathways in cancer include many oncogenic pathways that can modulate cellular activities, including cell proliferation, differentiation, angiogenesis, apoptosis, and cell cycle progression. Due to their pleiotropic nature, retinoids can alter many signaling pathways involved in cancer initiation and progression. There are several distinct binding proteins through which retinoids exert their effects, such as retinol binding protein (RBP), stimulated by retinoic acid gene 6 (STRA6), cellular retinol binding protein (CRBP), cellular retinoic acid-binding protein (CRABP), and nuclear receptors such as retinoic acid receptors (RARs) and retinoid X receptors (RXRs). These receptors are present in the extracellular and intracellular compartments and transfer retinol from ingested food to target cells. First retinol binds with RBP and translocates into the cell by STRA6 mediated endocytosis or diffusion [49]. In lung cancer, STRA6 is found to be frequently upregulated, but the mechanism is poorly understood.

Upon entry into the cell, retinol binds CRBP, and the retinol dehydrogenase (RDH) reversibly oxidizes retinol to retinal. CRBP is a key component of the retinoid pathway and is dysregulated in lung adenocarcinoma. High expression of CRBP is associated with upregulation of the EGFR/Akt/Erk pathway whereas treatment with RA inhibits EGFR/Akt/Erk leading to inhibition of cell growth [11,42,50,51]. CRABP mediates subsequent RA transportation from the cytosol to the nucleus. The two isoforms of CRABP, CRABP-I, and CRAB-II have a high affinity for RA. Favorskaya et al. reported that CRABP-II is suppressed in NSCLC [52]. It has been documented that CRABP-I mediates non-canonical RAR leads to membrane signal-free initiation of ERK1/2 by RA [53]. In some cancers including lung cancer, CRABP-I acts as a tumour suppressor while others demonstrate an association between CRABP-I and cancerous growth and reduced survival [52,54,55]. Therefore, targeting retinoid receptors and their downstream signaling pathways could inhibit lung cancer growth and promote apoptosis of lung cancer cells.

RAR β induces cell cycle arrest and apoptosis in a RAR α -dependent manner. RA-bound RAR α binds to the *cis*-acting retinoic acid response element (RARE) in the RAR β promoter and recruits various activator proteins that upregulate RAR β 2 gene expression [6]. RAR β mediated transactivation and expression of target genes is responsible for cancer cell apoptosis and differentiation. RAR β 2 is found to be downregulated in bronchial biopsy specimens of heavy smokers, and cell lines of the liver, breast, and head and neck cancer [56–59]. RARs are also substrates for protein kinase C (PKC), protein kinase A (PKA), cyclin-dependent kinase 7 (CDK7), and p38 [60–63]. Srinivas et al. demonstrated that Akt phosphorylates the DNA-binding domain of RAR α at Ser96, leading to inhibition of RAR α transactivation [64]. Mutation of Ser96 suppressed RAR α phosphorylation and transactivation, leading to retinoid resistance in NSCLC [64]. Karamouzis and colleagues developed a ‘switch on/off model to represent the crosstalk between retinoid receptors and other signaling molecules during early stage bronchial carcinogenesis [65]. It has been shown that RXR selective agents inhibit the activity of AP-1 and promote anti-proliferative activity in respiratory epithelium. Further, RAR β and RXR α can crosstalk with other nuclear hormone receptors such as PPAR γ leading to apoptosis [65].

RA inhibited EGFR expression at the transcriptional level by targeting the EGFR promoter leading to inhibition of lung cancer cell growth [66]. RA arrests TKI resistant NSCLC cells in the G0/G1 phase of the cell cycle by altering the expression of GATA6 and inhibits the activation of two important pathways involved in lung cancer progression namely EGFR and Wnt signaling to overcome TKI resistance [11]. GATA6 induced the downregulation of EGFR and CTNNB1 by binding to their promoter regions and also activated the Wnt inhibitor (FZD2) leading to inhibition of the Wnt signaling pathway. G-protein coupled receptor family C group 5 member A (GPRC5A), an RA-inducible gene is prominently expressed in normal lung tissue. In addition, the physical interaction of GPRC5A with EGFR is essential for EGFR inhibition [67,68]. Suppression of GPRC5A causes EGFR dysregulation, which is associated with lung tumorigenesis [69]. Overall, retinoids have a potent suppressive effect on signaling pathways that are aberrant in lung cancer, as summarized in Fig. 3.

3.3. Retinoids as an epigenetic modulator in lung cancer

Aberrant methylation of CpG islands in gene promoters is a primary epigenetic mechanism for transcriptional silencing of tumour suppressor genes in cancer [70–72]. Promoter hypermethylation of specific genes has the potential to be used to diagnosis and predict the risk for many types of cancers, including lung cancer [73]. Previous studies suggested that frequent aberrant methylation of the RAR β promoter silences RAR β expression during tumour progression, which indicated that RAR β is a tumour suppressor protein [74–76]. It has been shown that inhibitors of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) can re-express epigenetically silenced RAR β [22]. RA or the pan-HDAC inhibitor (panobinostat) showed moderate efficacy in NSCLC cells. However, combined treatment had synergistic effects on global histone H3 acetylation and differentiation. Also, the combined treatment of RA and panobinostat showed enhanced efficacy in upregulating p53 and p21CIP1/WAF1 proteins and downregulating phospho-ERK and phospho-AKT. Han et al. showed that combination therapy of RA or 9-*cis*-RA and HDAC inhibitors (SL142 and SL325) showed a significant reduction in single cell colony forming ability with increased apoptosis in NSCLC cells [24]. Moreover, combination treatment enhanced Bax expression and caspase-3 activity. These results indicate that RA combined with HDAC inhibitors is a promising treatment for lung cancer [24].

Virmani and colleagues reported a role for methylation of the RAR β P2 promoter in gene silencing of RAR β 2 and RAR β 4 in lung cancer, especially in SCLC [21]. In contrast, Dutkowska and colleagues did not find a correlation between RAR β gene expression and methylation, but RAR β gene expression was significantly downregulated. Adenocarcinoma and large cell carcinoma patients showed lower expression of RAR β as compared to squamous cell carcinoma patients, supporting the use of the differential expression of the RAR β gene as a prognostic biomarker to differentiate lung cancer subtypes [77]. A meta-analysis of 18 relevant studies that included 1871 participants suggested an increase in the frequency of RAR β hyper-methylation in NSCLC patients compared to non-malignant lung tissues. The RAR β gene of NSCLC patients who smoke was hypermethylated (2.46 fold) compared to nonsmokers [78].

Furthermore, another meta-analysis of 16 studies including 1780 lung cancer patients also observed RAR β hyper-methylation [79]. Interestingly, RAR β gene hypermethylation was again significantly higher in lung adenocarcinoma patients as compared to squamous cell carcinoma patients. Overall, both meta-analyses support a correlation between RAR β hyper-methylation and high risk of NSCLC [79]. It has been shown that treatment with glucocorticoids plus RA (GC/RA) in combination with epigenetic drugs (azacytidine and SAHA; A/S) enhanced growth inhibitory effect of A/S in MYC amplified lung cancer cells which supports a pro-differentiation action of GC/RA [80]. These findings suggest an epigenetic regulatory effect of retinoids as a combination drug during lung cancer treatment. Overall findings indicate that deregulated histone H3 acetylation and RAR β methylation have roles in lung cancer development and could be targeted to restore retinoic acid sensitivity to lung cancer patients.

3.4. Chemoresistance associated with retinoids in lung cancer

Cancer recurrence after treatment implies the dormancy of some cancer cells during the treatment period that reemerges later. This rare subpopulation of chemoresistant cancer cells is defined as cancer stem cells (CSCs) or tumour-initiating cells (TICs) [2]. CSCs possess similar properties to normal stem cells (self-renewal and multi-lineage differentiation) [81]. CSCs can sustain a tumour, undergo extensive proliferation, and mediate resistance against chemo- and radiation therapy in many cancers [2]. CSCs were first identified in acute myeloid leukemia, but further studies revealed their existence in other cancer types including cancer of the lung [82]. Vitamin A derived RA controls the expression and activity of many genes involved in cellular development and differentiation. In the past two decades, vitamin A and its natural and synthetic derivatives (retinoids) have been shown to regulate many cellular activities including proliferation, differentiation, and apoptosis [29,83,84]. Tumour-initiating cells (TICs) have great therapeutic importance due to their tumour-inducing potential and chemotherapy resistance.

Moro et al. described TICs in cisplatin-resistant NSCLC that express CD133 and CXCR4 [3]. The authors reported heterogeneity in TICs through *in-vitro* cell fate tracing systems. TICs were of two types: (1) a highly quiescent pool and (2) a slowly dividing subpopulation. The highly quiescent pool was enriched for CD133+/CXCR4- cells while the slowly dividing subpopulation was enriched for CD133+/CXCR4+ cells. Further, pre-treatment with RA neutralized cisplatin resistance; mainly in the slowly dividing CD133+/CXCR4+ subpopulation [3]. Similar results were observed in patient-derived xenografts. Treatment with RA in combination with cisplatin reduced the fraction of TICs and tumour metastasis [3]. Cells exhibiting aberrant retinol signaling show a high level of resistance to chemo- and radiation therapy [85].

Several clinical studies support that resistance to retinoids in lung cancer is mediated by repression of RAR β [13,21,58,59,86–90]. It was shown that RAR β 2 overexpression inhibits lung cancer cell proliferation as restores retinoid sensitivity to retinoid-resistant human lung cancer cells [13]. However, RAR β suppression has a modulatory effect on the expression of other retinoid receptors involved in retinoid resistance [91]. Petty and colleagues also observed hypermethylation of the RAR β P2 promoter in RA resistant lung cancer cells

leading to RAR β silencing [92]. RAR β 1' (RAR β isoform) is expressed in normal and RA-sensitive lung cells but not in RA resistant lung cancer cells or clinical lung cancer specimens. Transfection of RAR β 1' into RA resistant lung cancer cells with suppressed RAR β 2 restored RA sensitivity and mediated growth inhibition [92]. These results confirmed that loss of RAR β and RAR β 1' play a role in retinoid resistance in lung carcinogenesis. Interestingly, overexpression of exogenous nuclear retinoid receptors could also restore growth inhibitory effects of RA in lung cancer cells resistant to RA [19].

García-Regalado et al. revealed a new molecular mechanism responsible for RA resistance in lung cancer. They suggested that Akt pathway activation induces resistance to RA in lung cancer by promoting a RAR α -Akt interaction leading to activation of PI3k-Akt through transcription-independent mechanisms (Rac-GTPase activation) [51]. Overexpression of Akt or combination treatment of RA and a PI3k inhibitor suppressed the survival and invasion of lung cancer cells through overexpression of caspase-3 and RAR β 2 [51]. Similarly, inhibitors of the ERK pathway restored the effects of RA including inhibition of proliferation and migration in lung cancer cells. This suggests that targeting PI3k-Akt or ERK in combination with RA treatment is a promising therapeutic strategy [42]. Zito et al. found that RA treatment inhibits the growth of tyrosine kinase inhibitor (TKI) resistant NSCLC cells [11].

SCLC also demonstrate resistant to RA due to loss of RAR β expression and retroviral transduction of the RAR β gene re-sensitized SCLC cells to RA [16]. G1 phase arrest with downregulation of L-myc and cyclin-dependent kinase 2 (CDK2) and enhanced expression of CDK inhibitor p27Kip1 has been observed after RA treatment in RAR β overexpressed SCLC cells. In conclusion, repression of RAR α and RAR β has critical roles in retinoid intrinsic and acquired resistance in human lung cancer cells, which limits the use of retinoids as a therapeutic against lung cancer. Alternative clinical strategies are needed to overcome retinoid resistance such as the use of non-classical retinoids that have retinoid receptor-independent activity. The pathways involved in retinoids resistance in lung cancer are summarized in Fig. 4. The use of retinoids as a re-sensitizing agent for TKIs resistant lung cancer is summarized in Fig. 5.

3.5. Clinical significance of retinoids in lung cancer

Lung cancer is a significant cause of mortality and morbidity worldwide, with 1.38 million deaths annually [1]. The majority of patients are diagnosed at clinical stages III b and IV resulting in a survival rate of less than 5%. Natural and synthetic retinoids are well-known chemotherapeutic and chemopreventive agents and play a pivotal role in cancer cell differentiation, proliferation, and apoptosis. Hong and Itri in 1994 reviewed epidemiological evidence that found an inverse relationship between β -carotene and serum vitamin A levels and cancer incidence [93]. Wolbach and Howe published the first preclinical study on vitamin A in cancer in 1925 [94]. Their study suggested that rodents with vitamin A deficiency undergo squamous metaplasia of lung epithelia that was overcome after repletion with vitamin A [94]. Similar types of metaplastic changes are observed in smokers. Experimental chemopreventive studies on retinoids in animal models revealed a mutation suppressive role for retinoids [95]. According to National Cancer Institute population-based trials, β -carotene and retinoids were promising anticancer agents in the early 1980s [96].

Studies also suggested that deficiency of vitamin A promoted preneoplastic changes in the bronchial epithelium which was associated with a higher risk of lung cancer development.

Initial clinical trials supported retinoids in the treatment of aerodigestive tract cancers. High dose vitamin A was given as adjuvant therapy to 307 patients with stage I NSCLC. Upon curative surgery, patients were randomly assigned to retinol palmitate (300,000 IU orally daily for 12 months) or the control group. Forty-six months of observation revealed a 37% rate of new primary tumour recurrence in the treated group compared to 48% in the control group. This study concluded that vitamin A could reduce the number of new primary tumours in tobacco-related stage I lung cancer patients [97]. A program on vitamin A to prevent cancer in asbestos-exposed workers (Wittenoom Gorge between 1943 and 1966) was initiated by Musk et al. [98]. Selected workers were supplemented with vitamin A (either synthetic β -carotene or retinol) from June 1990 to May 1995 with no smoking whereas the control groups were not supplemented with vitamin A. A total of 12 cases of lung cancer was observed in the vitamin A-supplemented group compared to 30 cases in the control group, suggesting vitamin A supplementation can protect against lung cancer malignancy in asbestos-exposed workers [98].

Lippman et al. conducted a National Cancer Institute Intergroup randomized, double-blind, phase III trial of placebo or isotretinoin (13-*cis*-RA; 30 mg/day) for three years in 1166 patients with stage I NSCLC [99]. The trial suggested that isotretinoin treatment did not improve the mortality of NSCLC patients. Isotretinoin was found to benefit only never smokers and harmed current smokers [99]. A double-blind, randomized clinical trial, the α -Tocopherol, β -Carotene Cancer Prevention Study (ATBC), was performed in which doses of β -carotene (20 mg/day), α -tocopherol (50 mg/day) or both were daily supplemented to 29,133 male smokers [100]. The β -Carotene and Retinol Efficacy Trial (CARET) enlisted 18,314 men and women at high risk of lung cancer for supplementation with placebo or combined doses of β -carotene (30 mg/day) and retinyl palmitate (25,000 IU/day) for approximately four years [101]. In addition, the double-blind, randomized trial of β -carotene (50 mg on alternate days) supplementation among 22,071 male physicians (40–84 years of age) for 12 years was conducted by the Physicians' Health Study (PHS) [102]. These clinical trials revealed that retinoids were effective in reducing tumour induction and mortality in non-smokers and former smokers. However, retinoids increased the incidence of lung cancer in smokers, which was further supported in the EUROSCAN study [103].

Bushue et al. suggested that retinoids could cause bio-activation of cigarette carcinogens leading to an enhanced risk of lung cancer in smokers. A combination study on the effects of vitamin and mineral supplementation on lung cancer mortality among 29,584 healthy adults from Linxian, China suggested that combinations of vitamins and minerals failed to reduce lung cancer-related mortality in the selected population [104]. Another study based on cyclophosphamide and biotherapeutic compounds such as retinoids were conducted on 28 patients with advanced NSCLC and low-performance status with no previous record of surgery, chemotherapy or radiation [105]. Patients received a daily dose of (bromocriptine, cyclophosphamide, melatonin, retinoids, somatostatin, and vitamin D) every day for an indefinite period until unacceptable toxicity. This combined treatment was associated with

improved survival and quality of life in advanced NSCLC patients with low-performance status [105].

RA and 13-*cis*-RA are the most active metabolites of vitamin A and both have effects on cell growth, differentiation, and apoptosis. Use of RA alone for treatment of twenty-eight metastatic patients exhibited minimal anti-lung cancer activity with an acceptable toxicity profile [43]. During a randomized phase II multi-center trial, combined treatment of interferon (IFN)- α and RA improved the survival rate of lung cancer patients who were treated with conventional cancer therapeutics [106]. However, the combined treatment of IFN- α and RA in twenty-nine un-resurrected NSCLC patients showed only modest anticancer activity with moderate/severe hypertriglyceridemia and low toxicity in patients (Athanasiadis et al.1995). Combined treatment of RA and cisplatin or paclitaxel enhanced the median progression-free survival of advanced NSCLC patients [107]. RA shows less bioavailability after prolonged periods of administration. Thiruvengadam and colleagues treated 20 stage IIIB and IV NSCLC patients with an interrupted schedule of RA alone or in combination with cisplatin-VP 16 [108]. Ten patients out of 19 had a partial response whereas four patients had a minor response with neutropenia (most common acute toxicity). The average median survival rate was 25.5 weeks [108].

Further, the nano-encapsulation of RA using poly (lactic-co-glycolic) acid (PLGA) polymer has been successful in overcoming bioavailability related issues during treatment [109]. Zhang et al. formulated RA loaded PLGA nanoparticles with CD133 aptamers that have targeting efficiency for CD133+ lung cancer initiating cells. This PLGA nano-formulation showed higher inhibitory effects toward CD133+ lung cancer TICs as compared to non-targeted PLGA nanoparticles and RA [109]. Therefore, an interrupting schedule or nano-formulation of RA in combination with traditional chemotherapeutics may be an option to overcome bioavailability related issues in lung cancer therapy.

A phase II randomized clinical trial of 13-*cis*-RA with or without α -tocopherol was conducted by Kelly et al. on subjects with a high risk of lung cancer [110]. Bronchial biopsy histology showed only minor effects of 13-*cis*-RA and α -tocopherol did not alleviate 13-*cis*-RA toxicity [110]. Pillai and colleagues conducted a phase II trial to evaluate the effects of 13-*cis*-RA, interferon- α , and paclitaxel combination therapy on 37 recurrent SCLC patients [111]. Three patients showed a partial response with 13-*cis*-RA, whereas five patients had stable disease. The median progression-free survival and median overall survival was 2 and 6.2 months, respectively.

Bexarotene (targretin) is approved for the treatment of cutaneous T-cell lymphoma and is an agonist of the retinoid-X-receptor and inducer of CYP3A4-mediated metabolism. Bexarotene induces cell differentiation and apoptosis leading to the prevention of drug resistance in cancer cells including solid tumours. Interestingly, bexarotene showed good tolerability and disease stabilization in NSCLC patients in phase I trials [112,113]. Bexarotene also demonstrated excellent tolerability in lung cancer patients when used in combination with cisplatin or vinorelbine with an improved patient survival rate. Some common side effects observed during treatment were anemia, asthenia, hyperlipidemia, leukopenia, nausea, and vomiting [114]. Another, phase II trial on combined bexarotene and

gemcitabine or carboplatin found that stage IIIB or stage IV NSCLC patients benefited from the combination with higher tolerance to treatment and improved progression time and overall survival [115]. A pharmacokinetic study of bexarotene with established chemotherapeutic drugs (such as paclitaxel, carboplatin, cisplatin, and vinorelbine) suggested drug-drug interactions during bexarotene co-administration. Co-administration of atorvastatin or fenofibrate with bexarotene suggested that co-administration of atorvastatin controls bexarotene-mediated hypertriglyceridemia more effectively than fenofibrate in NSCLC patients [111,112]. The SPIRIT I and SPIRIT II (randomized phase III trials of bexarotene) failed to improve the survival rate of chemotherapy-naïve advanced or metastatic NSCLC patients [47,116]. However, a subpopulation of bexarotene treated NSCLC patients showed high-grade hypertriglyceridemia and an improved survival rate. Both SPIRIT I and SPIRIT II suggests that triglyceride induction could be a biomarker for bexarotene treatment of lung cancer patients. Overall, these findings suggest that bexarotene could be safely added to platinum-based chemotherapeutics for lung cancer treatment and warrant further clinical evaluations. Clinical use of retinoids as combinatorial drug therapeutics is summarized in Table 2.

4. Conclusion and future perspective

Lung cancer is one of the most aggressive and life-threatening forms of cancer worldwide. Improvement in the treatment of lung cancer is an urgent human health need. Retinoids have various anticancer properties including anti-proliferative, anti-invasive, anti-migratory, anti-angiogenic, antimetastatic, and pro-apoptotic effects through modulating aberrant oncogenic signaling pathways. Retinoids also regulate the survival and differentiation of tumour-initiating cells or cancer stem cells. Studies highlight the potential of retinoids in the epigenetic regulation of oncogenes and tumour suppressor genes in lung cancer. However, despite this preclinical promise, retinoids have failed to show significant clinical utility as single agents for the treatment of naïve and drug-resistant lung cancer. One possible reason for single agent failure could be poor solubility and low bioavailability of retinoids, which may be overcome through retinoid nano-formulation. Retinoids can be used as a combination therapeutics for the resensitization of TKIs resistant lung cancer. The retinoid resistance in lung cancer can be overcome by cotreatment of drugs which target the cancer stem cells. The best way to harness these powerful anticancer agents to treat lung cancer is likely combination strategies with conventional and targeted therapies to improve efficacy and limit toxicity.

Acknowledgements

The authors thank (a) MHRD and National Institute of Technology, Rourkela, Odisha, India for providing laboratory and fellowship to Surya Kant Tripathi; (b) Department of Science and Technology, Science and Engineering Research Board (DST, SERB) (Grant Number: ECR/2016/000792) for providing fellowship to Munmun Panda; (c) Department of Science and Technology, Odisha, India (Grant number: 1201) for providing fellowship to Kamal Pandey and (d) National Institutes of Health grants R01-CA211875 and R03CA223709 and a Reach Grant from the Alex's Lemonade Stand Foundation to MJS, and (e) Konkuk University, Seoul, South Korea for providing support through KU Brain Pool Program to KRRR.

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Fig. 1. Chemical structures of natural (1–6) and synthetic (7–8) retinoids.

Retinol (1), Retina (2), Retinoic acid (3), 13-*cis* Retinoic acid (4), 9-*cis* Retinoic acid (5), 14-Hydroxy-retro-retinol (6), Bexarotene (7), Fenretinide (8).

Fig. 2.

Model showing the anti-angiogenic and anti-metastatic effect of retinoic acid (RA) as a combinatorial drug in lung cancer progression. RA promotes angiogenesis and metastasis in lung cancer *via* activation of the extracellular-signal-regulated kinase (ERK) pathway. Activation of the ERK pathway upregulates hypoxia-inducible factor- α (HIF- α), activator protein-1 (AP-1), and activator protein-2 (AP-2) gene expression which favors the upregulation of many factors involved in angiogenesis and metastasis such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and matrix metalloproteinases (MMPs). However, activation of the ERK pathway alleviates the expression of MMPs inhibitors such as TIMP metalloproteinase inhibitor (TIMP). Treatment with a combination of RA and ERK inhibitors drastically overcomes this effect of RA resulting in inhibition of angiogenesis and metastasis in lung tumours. Bexarotene also inhibits angiogenesis and metastasis of lung tumours *via* ERK pathway inhibition. The high concentration of liposome encapsulated RA inhibits angiogenesis and metastasis in lung tumours *via* ERK pathway inhibition.

Fig. 3. Retinoid signaling pathways leading to lung cancer stem cell (CSC) differentiation and cell cycle arrest.

Retinol binds with retinol binding protein (RBP) and translocates into lung cancer cells by retinoic acid gene 6 (STRA6) receptor mediated endocytosis or diffusion. Inside the cell, it interacts with cellular retinol binding protein (CRBP) and oxidizes into RA by enzyme retinol dehydrogenase (RDH). RA inhibits the EGFR/Wnt pathways *via* activation of transcription factor GATA-binding factor 6 (GATA6). GATA6 upregulates the expression of (SEPTB), mucin4 (MUC4), frizzled class receptor 2 (FZD2) and downregulates the expression of epidermal growth factor receptor (EGFR), catenin beta (CTNNB), and cyclin D1. Importantly, EGFR and CTNNB downregulation inhibit the activation of the Wnt signaling pathway; however, downregulation of cyclin D1 promotes lung CSC differentiation and cell cycle arrest. Furthermore, RA alone may promote cancer cell survival, proliferation, migration, and invasion *via* activation of PI3-Akt/ERK pathways. Combinatorial treatment of RA with PI3K/ERK inhibitors represses these pro-cancer effects of RA in lung tumours.

Fig. 4. Retinoid resistant pathways in lung cancer.

Lung tumour inducing cells (TICs) or cancer stem cells (CSCs) positive for CD133⁺/CXCR4⁺ are sensitive to retinoids *via* inhibition of the PI3K-Akt pathway. However, CD133⁺/CXCR4⁻ lung TICs/CSCs exhibit resistance to retinoid treatment. How CD133⁺/CXCR4⁻ lung TICs/CSCs modulate the PI3K-Akt pathway during retinoid treatment and the potential to target this pathway is an active area of research.

Fig. 5. Re-sensitizing effect of retinoids as a combinatorial drug in TKIs resistant lung cancer. Combinatorial treatment of retinoids with TKIs drugs in TKIs resistance lung cancer cells promotes the activation of a transcription factor named GATA-binding factor 6 (GATA6). Activation of GATA6 inhibits the activation of EGFR/Wnt signaling pathways and favors the association of retinoid X receptors (RXR), retinoic acid receptor β (RAR β), and cellular retinoic acid-binding protein-2 (CRABP2). This complex inhibits the proliferation and

promotes the differentiation of lung tumour cells *via* inhibiting activating protein-2 (AP-2), which result in re-sensitization of TKIs resistant lung cancer cells.

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Table 1

Alterations in retinoid receptors associated with retinoid resistance.

Sl. No	Model system	Retinoic acid receptors	Overcoming strategy	Outcome	References
1.	Calu-1 cell line	Does not express RAR β and thus RA resistant	Transfection of pMARKCD7D5 vectors containing RAR α , RAR β , RXR α , and RAR γ	Sensitized cancer cells towards RA after transfection with RAR α , RAR β or RXR α but not RAR γ	[19]
2.	<i>In-vitro</i> and <i>in-vivo</i> model	Loss of RAR β 2 and RAR β 4 expression due to hypermethylation of promoter region	5-aza-deoxycytidine treatment	Restored RAR β expression and inhibited metastatic lung cancer	[20,21]
3.	H1792 cell line	Expresses endogenous RXR α but does not activate DR1 RXE after treatment with 9- <i>cis</i> -RA	Transfection of exogenous RXR α	Sensitized cells towards 9- <i>cis</i> -RA to activate DR1 RXE	[19]
4.	A549, HCC827, and NCI-H460 cell lines	RAR β 2 downregulated thus shows less sensitivity towards ATRA	Combination of panobinostat + ATRA	Showed enhanced inhibitory effects by synergistic H3 acetylation	[22]
5.	Clinical trial	RAR β loss in smokers which is a pre-neoplasia symptom	9- <i>cis</i> -RA treatment	Restored RAR β expression in bronchial epithelium in former smokers and reduction of metaplasia	[23]
6.	<i>In-vitro</i> model	RAR α , RAR β , and RXR α , RXR β expressions unchanged after treatment	Treatment with ATRA or 9- <i>cis</i> -RA along with HDAC inhibitors SL142 or SL325	Induced enhanced antitumor activity	[24]
7.	A/J mouse model	Upregulation of RAR β expression after treatment	9- <i>cis</i> -RA treatment	Lowered tumour multiplicity and tumour incidence	[25]
8.	<i>In-vitro</i> study	RAR β and RXR β upregulated post treatment	Combination treatment of ATRA and 9- <i>cis</i> -RA	Showed synergistic inhibition of lung cancer cell line	[26]
9.	Calu-6, H460, H292, SK-MES-1, and H661 cell lines	RAR β expression induced upon treatment with ATRA only in Calu-6, and H460 cell line	ATRA treatment	Induced apoptosis in Calu-6, and H460 cell line while little efficacy in other cell lines	[27]
10.	<i>In-vitro</i> study	Treatment in H1703 cells upregulated RAR β expression which had no basal expression of RAR β	Treatment with ATRA or 13- <i>cis</i> -RA followed by 4-HPR	Synergistic growth inhibitory effect	[28]

Table 2

Clinical use of retinoids as combinatorial drug therapeutics.

Retinoids	Combination	Consequence	Stage of study	Reference
All- <i>trans</i> retinoic acid	Somatostatin, melatonin, vitamin D, bromocriptine, and cyclophosphamide	Overall survival 12.9 months	Stage IIIB or stage IV	[105]
	C-phycocyanin	Synergistic	<i>In-vitro</i> and <i>in-vivo</i>	[117]
	Arsenic trioxide	Synergistic	<i>In-vitro</i>	[118]
	Paclitaxel and cisplatin	Progression-free survival 8.9 months	Stage IIIB, stage IV	[107]
	c-myc antisense DNA	Synergistic	<i>In-vitro</i>	[119]
	IFN- α	Overall survival eight months	Phase II trial	[120]
	Bexarotene, capecitabine, and docetaxel	Survival benefit	Stage IIIB and IV	[121]
	PLGA nanoparticles with CD133 aptamers	Enhanced inhibitory effect	<i>In-vitro</i>	[109]
	Gemcitabine, Carboplatin	Increased overall survival	Stage IIIB, stage IV	[115]
	Carboplatin, Paclitaxel	May benefit a segment of first-line NSCLC patients	Phase III trial	[116]
13- <i>cis</i> -retinoic acid	Paclitaxel, vinorelbine	Enhanced inhibitory effect	<i>In-vitro</i> and <i>in-vivo</i>	[122]
	Cisplatin, vinorelbine	Overall survival 8.7 months	Phase I/II trial	[114]
	Cisplatin, vinorelbine	No survival benefits in advanced NSCLC	Phase III trial	[47]
	IFN- α , paclitaxel	Overall survival 6.2 months	Phase II study	[111]
	α Tocopherol	Reduced bronchial epithelial cell proliferation	Previous smokers	[123]
	Interleukin-2	Improves progression-free and overall survival	Phase II study	[48]
	Curcumin	Synergistic effect	<i>In-vitro</i> , <i>in-vivo</i>	[124]
	Etoposide, paclitaxel, cisplatin	Enhanced inhibitory effect	<i>In-vitro</i>	[125]
	Retinyl Palmitate	Overall survival 9.1 months	Phase II study	[126]
	5 FU, Cisplatin, IFN- β ,			