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REVIEW

Retinoids: novel immunomodulators and tumour-suppressive agents?

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Retinoids play important roles in the transcriptional activity of normal, degenerative and tumour cells. Retinoid analogues may be promising therapeutic agents for the treatment of immune disorders as different as type I diabetes and systemic lupus erythematosus. In addition, the use of retinoids in cancer treatment has progressed significantly in the last two decades; thus, numerous retinoid compounds have been synthesized and tested. In this paper, the actual or potential use of retinoids as immunomodulators or tumour-suppressive agents is discussed.

Abbreviations

13-cRA, 13-*cis*-retinoic acid; 9-cRA, 9-*cis*-retinoic acid; AF-1, activation function 1; AML, acute myeloid leukaemia; APL, acute promyelocytic leukaemia; ATRA, all-trans retinoic acid; CD347, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; CML, chronic myeloid leukaemia; DBD, DNA-binding domain; HBV, hepatitis virus B; HCC, hepatocellular carcinoma; HCV, hepatitis virus C; HPR, *N*-(4-hydroxyphenyl) retinamide; LBD, ligand-binding domain; NGF, nerve growth factor; NIS, sodium iodide symporter; NOD/SCID, non-obese diabetic/severe combined immunodeficient; NR, nuclear receptor; PGZ, pioglitazone; PML, promyelocytic leukaemia; PRAME, preferentially expressed antigen in melanoma; RA, retinoic acid; RAR, retinoid acid receptor; RGZ, rosiglitazone; RIG-I, retinoic acid inducible gene I; RXR, retinoid X receptor; SLE, systemic lupus erythematosus; T1D, Type 1 diabetes; Tg, thyroglobulin

Introduction

The retinoid system controls the expression of hundreds of genes, including transcription factors, enzymes, structural proteins, cell-surface receptors, neurotransmitters, neuropeptide hormones and growth factors (Balmer and Blomhoff, 2002). The observation that retinoic acid (RA) has distinct physiological functions during embryonic development and adult life has led to the concept that the retinoids may play important roles in the transcriptional activity of normal, degenerative and tumour cells (Gonlugur and Gonlugur, 2007). Both experimental and clinical evidence suggests that retinoid analogues may be promising therapeutic agents for the treatment of several immune disorders, such as type I diabetes (T1D) (Wasserfall and Atkinson, 2009) and systemic lupus erythematosus (SLE) (Kinoshita *et al.*, 2010). Moreover, the use of retinoids in cancer treatment has progressed significantly in the last two decades, and numerous retinoid compounds have been synthesized and tested. Among them, the all-trans RA (ATRA), 9-*cis*-retinoic (9-cRA) and 13-*cis*-RA (13-cRA) have repeatedly demonstrated their anti-cancer efficacy *in vivo* (Freemantle *et al.*, 2003; Altucci *et al.*, 2005; Dragnev *et al.*, 2007).

The retinoid system has been extensively described elsewhere (Mangelsdorf and Evans, 1995; Chambon, 1996; Bastien and Rochette-Egly, 2004). Briefly, RA takes part in several biological activities, such as embryogenesis, growth, differentiation, proliferation, bone formation, vision and metabolism (Sun and Lotan, 2002). RA effects are mediated by activating ligand-regulated transcription

factors belonging to nuclear hormone receptors (NRs) superfamily.

NRs are composed by a series of conserved domains. Their modular structure reveals distinct functional domains, including an N-terminal activation function 1 (AF-1), the DNA-binding domain (DBD), the hinge region and a C-terminal ligand-binding domain (LBD), which contains a ligand-dependent AF-2 domain (Freedman, 1999).

Retinoids act through two types of NRs, the RA receptors (RARs) and the retinoid X receptors (RXRs) (Kastner *et al.*, 1997). Each type has three different isotypes (α, β and γ)(Chambon, 1996). In order to work, RAR/RXR form a heterodimer (McKenna and O'Malley, 2002). In the absence of ligand, RARs and thyroid hormone are complexed with co-repressor protein. After ligand binding to these receptors, the conformational change in the LBD induces co-repressors to dislodge and co-activators to bind, leading to a number of downstream events finally resulting in the up- or down-regulation of gene expression.

The extent and duration of RA's action on target genes depend on multiple regulatory mechanisms, including the synthesis and degradation of RA, phosphorylation and degradation of the RARs, recruitment of different chromatin remodellers, and cellular processes involved with the transport, diffusion and cellular uptake of retinoids (Bastien and Rochette-Egly, 2004). Altogether, this complex machinery determines the amount of ligand available for receptor occupancy in target tissues. It also sets up a pre-receptor system, which directs the timing and tissue-specificity of RA signalling.

Retinoids act as immunomodulators

Retinoids are required for the maintenance of the immune system, as they are important immunomodulators. The tight relationship among senescence, immunity and retinoid levels has provided evidence of the crucial role of RAs in regulating immune activity. Major age-related changes in immunity include a progressive, age-dependent decline in the total number of T cells along with a progressive increase in effector/cytotoxic CD8+ T-cell subset producing pro-inflammatory cytokines such as IL-2, IFN-γ and TNF-α (Gupta *et al.*, 2004; Sansoni *et al.*, 2008). These changes have led to the hypothesis that immunosenescence is driven by a chronic elevated perceived antigenic load. This, in turn, may promote unremitting inflammatory and/or autoimmune processes negatively correlated with human longevity (Zanni *et al.*, 2003). Indeed, vitamin A levels are consistently elevated in healthy centenarians, suggesting a protective role in immune system maintenance by retinoids (Mecocci *et al.*, 2000; Basile *et al.*, 2003; Franceschi and Bonafe, 2003; Polidori *et al.*, 2007). According to this, a large body of experimental studies have suggested several molecular mechanisms underlying the beneficial effects of retinoids in immune system disorders. In particular, the identification of a new T helper cell population, namely T_h17, has further expanded the potential use of retinoids as immunomodulators. T_h17 cells are critical for the enhancement of host protection against extracellular bacteria and fungi. Furthermore, it has emerged that they can act as mediators in autoimmune disease (Honkanen *et al.*, 2010; Schuhmann *et al.*, 2010). On the other hand, regulatory T cells (T_{reg}) maintain immune tolerance and protect against excessive T_h effector activity and autoimmune pathology. T_{reg} cells are characterized by the expression of a DNA-binding protein, that is, the forkhead-winged helix transcription factor family member FOXP3+, which is induced by TGF-β in the presence of IL-2. This process is significantly up-regulated by RA, which enhances T_{reg} expansion and inhibits T_h17 cell differentiation (Mucida *et al.*, 2009). FOXP3+ T_{reg} cells secrete IL-10, which suppresses aggressive effector T cell activation (Ochs *et al.*, 2009). This emerging scenario has revealed retinoids as important regulators in T_h1/T_h17 versus T_h2 balance. Indeed, vitamin A deficiency acts in favour of T_h1/T_h17 cells, while adequate levels of vitamin A promote the development of T_{reg} cells responsible for self-tolerance (Pino-Lagos *et al.*, 2008; Ertesvag *et al.*, 2009). T_{reg} cells represent 5–10% of peripheral CD4+ T cells in naïve mice and humans. Chronic ablation of T_{reg} cells in adult healthy mice leads to death within 3 weeks, underlying the pivotal role of T_{reg} cell-mediated suppression in preventing autoimmune pathology (Lu and Rudensky, 2009). In the intestine, an altered balance between inflammatory and suppressive immunity can jeopardize mucosal homeostasis and destroy the integrity of the mucosal barrier. RA-induced FOXP3+ T_{reg} cells play a key role in maintaining the steady state of tolerance towards innocuous antigens, thus preventing excessive, self-destructive immune responses and the development of inflammatory bowel disease and autoimmunity (Strober, 2008).

Besides the TGF-β–driven T_{reg} expansion in peripheral tissues, RA enhances the production of T_{reg} cells induced by antigen-presenting dendritic cells in the gut-associated lymphoid tissue and small intestinal lamina propria. RA also up-regulates gut-homing receptors expression in T_{reg} cells and B lymphocytes. Effector and memory T cells exhibit plasticity in their homing commitment: skin-homing T cells can become gut-homing T cells and *vice versa*, according to RA stimulation. B cells also exhibit plasticity in their homing commitment and can either acquire or loose gut-homing potential when reactivated with or without RA (Mora *et al.*, 2008). Both TGF-β and RA are actively produced by the intestinal epithelium and play a pivotal role in mucosal epithelial cell differentiation and in maintaining the integrity of its barrier function. For instance, TGF-β and RA positively regulate secretory IgA production, actively promoting IgA class switching.

The immunomodulatory activity of vitamin A metabolites, including RA, has been extensively documented in several inflammatory and autoimmune diseases. In NZB/W mice, ATRA treatment decreased anti-DNA antibody production, recovered partially proteinuria and increased overall survival. ATRA effects resided in inhibiting mRNA expression in CD4+ cells of pro-inflammatory cytokines such as IL-2 and IFN-γ and increasing renal TGF-β expression (Perez de Lema *et al.*, 2004; Nozaki *et al.*, 2005). Recently, retinoid treatment has been successfully employed in patients suffering from lupus nephritis, a major manifestation of SLE (Kinoshita *et al.*, 2010). Moreover, epidemiological studies suggest that either vitamin A or D may be beneficial in the treatment of patients with high risk of type 1 diabetes (T1D) (Wasserfall and Atkinson, 2009). ATRA is able to prevent overt develop-

ment of T1D animal models (Van *et al.*, 2009). In particular, the ATRA treatment markedly suppresses the transfer of diabetes by diabetogenic splenocytes in non-obese diabetic/severe combined immunodeficient (NOD/SCID) recipient mice and significantly delays progression to T1D in a 'late prevention' protocol, in which ATRA was administered i.p. to 10-week-old NOD mice (Van *et al.*, 2009).

Administration of retinoids may also be effective in preventing *diabetic neuropathy*, one of the most common complications of diabetes mellitus (Leinninger *et al.*, 2004; Said, 2007). In human and experimental models of diabetes, decreased serum levels of nerve growth factor (NGF) have been consistently related to the severity of neuropathy (Faradji and Sotelo, 1990; Ordonez *et al.*, 1994). RA would exert a supportive effect in axonal growth and neuronal survival, through transcriptional activation of neurotrophin genes, such as NGF and its receptor (Wion *et al.*, 1987; Corcoran and Maden, 1999; Ahlemeyer *et al.*, 2000; Balmer and Blomhoff, 2002). Furthermore, RA has been shown to have a synergistic neuroprotective effect along with NGF on intracellular pathways associated with neuronal growth and neuronal survival (Mey and Rombach, 1999; Ahlemeyer *et al.*, 2000; Plum *et al.*, 2001). RA also promotes functional regeneration of sensory axons in the spinal cord (Wong *et al.*, 2006). This effect could be a consequence of the different sensitivity to RA of the injured nerve compared with the undamaged nerve. Indeed, a damaged nerve can increase the RARα, RARβ and RARγ concentrations locally (Zhelyaznik and Mey, 2006). Because RA exerts most of its biological effects by interacting with RAR and RXR families, the injured nerves could increase NGF expression more than in undamaged nerves. Moreover, administration of RA prevents the drastic depletion of NGF in diabetic mice (Arrieta *et al.*, 2005). The significant increase in NGF in serum and nerves of diabetic mice after the administration of RA, suggests its potential use in the treatment of diabetic neuropathy (Hernandez-Pedro *et al.*, 2008).

Retinoids act as tumour-suppressive agents

Signalling through RAR and following activation of RAR, target genes induce arrest in proliferation, differentiation and apoptosis in a wide variety of cell types (Lawson and Berliner, 1999; Collins, 2002; Evans, 2005). There is increasing evidence that defects in RAR signalling are implicated in cancer. For example, loss of RARβ expression is involved in the pathogenesis of several haematological malignancies, as well as in progression of solid tumours such as breast and lung (Altucci and Gronemeyer, 2001; Freemantle *et al.*, 2006). Thus, molecules able to restore the retinoid system activity may have tumour-suppressive effects.

However, additional factors seem to be involved in the regulation of the RA system activity, as co-repressors expression changes. In contrast with the general assumption that ligand-dependent co-repressors of RAR signalling are not expected to increase in cancer, 'preferentially expressed antigen in melanoma' (PRAME) expression is up-regulated linearly with disease progression (Epping *et al.*, 2005). In solid tumour cell lines, PRAME favours proliferation rather than differentiation by hampering cellular responses to retinoids. These observations underline the complexity of the RA system and highlight the crucial role of RA signalling in tumour cell proliferation.

PRAME is over-expressed in several human malignancies, including acute and chronic leukaemias, medulloblastoma, non-small cell lung carcinoma, head and neck cancer, renal carcinoma, multiple myeloma, and sarcomas (Ikeda *et al.*, 1997; van Baren *et al.*, 1998; Oberthuer *et al.*, 2004). In contrast to solid tumours, in which increased PRAME expression is associated with poor outcomes (Oberthuer *et al.*, 2004), controversial data have emerged in haematological malignancies. Increased levels of PRAME expression have been observed in chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML) (van Baren *et al.*, 1998; Steinbach *et al.*, 2002; Radich *et al.*, 2006). PRAME is expressed in 22–62% of unsorted bone marrow or peripheral blood samples from CML patients and in 25–62% of paediatric AML cases (van Baren *et al.*, 1998; Steinbach *et al.*, 2002; Radich *et al.*, 2006; Paydas *et al.*, 2007). In patients with CML, PRAME expression increases linearly with disease progression (Radich *et al.*, 2006). Hypomethylation may contribute to PRAME increased expression in blast crisis during CML and AML (Roman-Gomez *et al.*, 2007; Ortmann *et al.*, 2008). In contrast with previous data, increased PRAME expression has been associated with better outcomes in paediatric AML, acute promyelocytic leukaemia (APL), and in adult AML with normal cytogenetics (Steinbach *et al.*, 2002; Santamaria *et al.*, 2008; 2009). Until recently the function of PRAME has remained unknown. Epping *et al.* (2005) have characterized PRAME as a ligand-dependent co-repressor of RARα, RARβ and RARγ signalling. They demonstrated that PRAME negatively regulates cellular responses to retinoids, thus favouring proliferation rather than differentiation in solid tumour cell lines. They also hypothesized that the polycomb group protein EZH2 may act synergistically with PRAME to block cell differentiation.

On the other hand, retinoids can trigger important downstream pathways, such as modulating transcription factor activity; for instance, growth-stimulating transcription factor AP1 is repressed by RA (Nicholson *et al.*, 1990). Among them, NF-κB has gained particular attention. NF-κB transactivates several genes involved in cell-cycle deregulation occurring in tumours and it represents a pivotal pro-survival factor for cancer cells. (Tai *et al.*, 2000; Factor *et al.*, 2001). Nevertheless, NF-κB activation can also lead to the up-regulation of pro-cancer types (Kucharczak *et al.*, 2003). In recent studies, it has emerged that retinoids exert their chemopreventive effect through NF-κB modulation, either inhibiting or activating it.

In a liver tumour rat model, the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (HPR) down-regulates NF-κB gene expression and inhibits its activity by enhancing NF-κB inhibitor IkB-α gene expression, leading to neoplastic lesion volume decreases (Simile *et al.*, 2005). Similarly, ATRA and 13-cRA dose-dependently inhibit NF-κB activation induced by alkylating chemical carcinogens in human cultured keratinocytes (Moon, 2007). In addition, 4-HPR induces apoptosis through NF-κB inhibition in a neuroblastoma xenograft model as well as ATRA in a gliobastoma cell line (Zhang *et al.*, 2007; Karmakar *et al.*, 2009). On the

other hand, a synthetic retinoid, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437), induces apoptosis in prostate cancer cells by inducing death receptors 4 and 5 (DR4 and DR5) expression throughout NF-κB activation. This is due to a rapid decrease in IkB-α, which allows NF-κB translocation in the nucleus leading to the transcription of pro-apoptotic genes (Jin *et al.*, 2005). A study in human melanoma cells demonstrated that CD347-induced NF-κB activation is mediated by RA inducible gene I (RIG-I) (Pan *et al.*, 2009). A suggested mechanism involved in IkB-α increased degradation, it is represented by IKKα kinase activation, which is associated with IKKα-enhanced binding to HSP90 (Farhana *et al.*, 2005). In addition, an adamantyl-substituted retinoid molecule, 3-Cl-AHPC, can activate both canonical and non-canonical NF-κB pathway in order to maximize the apoptotic effect (Farhana *et al.*, 2011). These findings may help to better understand the beneficial effects of retinoid administration in several malignancies.

APL is a subtype of acute myeloid leukaemia comprising 5–15% of all AMLs. APL is characterized by selective expansion of immature myeloid precursors blocked at the promyelocytic stage. APL is due to a translocation between chromosomes 15 and 17. The break in chromosome 15 disrupts the promyelocytic leukaemia (PML) gene encoding for a growth-suppressing transcription factor. The break in chromosome 17 interrupts the RARα gene, which regulates myeloid differentiation. Translocation of RARα gives rise to the chimeric genes PML-RARα and PLZF-RARα, which result in functionally altered receptors that act as constitutive repressors of transcription. As a result, cell differentiation is prevented and maturation of the myeloid cells is arrested at the promyelocytic stage (Grignani *et al.*, 1998; He *et al.*, 1998; Lin *et al.*, 1998). The introduction of ATRA completely revolutionized the APL management and outcome. ATRA induces differentiation, which activates RARs. This effect enhances the promyelocytes differentiation and hampers their proliferation (Warrell *et al.*, 1993). In APL, the PML/RARα chimeric gene inhibits RA-induced gene transcription and cell differentiation, although a supraphysiological ATRA concentration can overcome this inhibition and promote granulocytic differentiation. However, ATRA's effects on normal progenitor cells are dependent on cell phenotype and concentration (Collins, 2002). Whereas *in vitro* supraphysiological concentrations of ATRA shift haematopoiesis towards granulopoiesis, physiological concentrations increase proliferation and promote colony formation of a number of cell lineages (Zauli *et al.*, 1995).

Expanding the spectrum of haematological malignancies that may respond to ATRA remains a challenge. Interestingly, recent studies on *multiple myeloma* cells showed a potential clinical benefit from ATRA treatment. According to this, RXRα may form a heterodimer with PPARγ and bind to specific PP response elements in the promoter region of target genes to regulate transcription. Then, PPARγ/RXRα heterodimers can be activated by the ligation of PPARγ or RXRα ligands, but the combination of retinoids with PPARγ ligands and simultaneous ligation of both PPARγ and RXRα may be beneficial and may maximize their activation (Nolte *et al.*, 1998; Desvergne and Wahli, 1999). PPARγ can be found in tumour tissues including human breast cancer (Mueller *et al.*, 1998), lung cancer (Chang and Szabo, 2000; Roman, 2008), colon cancer (Sarraf *et al.*, 1998; Kitamura *et al.*, 1999), prostate cancer (Hisatake *et al.*, 2000; Smith and Kantoff, 2002), pancreatic cancer (Motomura *et al.*, 2000), as well as in human leukaemia and lymphoma cells (Asou *et al.*, 1999; Padilla *et al.*, 2002; Laurora *et al.*, 2003). Activation of PPARγ by its ligands has potential anti-neoplastic effects in these malignancies (Koeffler, 2003). Several studies have demonstrated that PPARγ is also expressed in human multiple myeloma cells and PPARγ ligands play a role in apoptosis of myeloma cells (Eucker *et al.*, 2004; Ray *et al.*, 2004). PPARγ ligands may therefore represent novel agents for human multiple myeloma. Recently, it has been shown that concomitant RXRα activation by ATRA enhances the inhibitory effects of PPARγ agonist rosiglitazone (RGZ) on myeloma cell proliferation, cell cycle, apoptosis and differentiation (Huang *et al.*, 2009). Therefore, the combination of RGZ and ATRA could be a useful therapy for human multiple myeloma.

Abnormalities in the expression and function of both RARs and RXRs also have an important role in influencing the growth of various epithelial malignancies. The development of *hepatocellular carcinoma* (HCC) is generally associated with chronic liver inflammation induced by a persistent infection of hepatitis viruses B (HBV) and C (HCV). Poor prognosis for HCC is due to the lack of effective therapeutic agents. RXRα is able to bind the enhancer element of HBV (Garcia *et al.*, 1993). The RARα gene is located near one of the integration sites of HBV and its expression is induced in HCC (Benbrook *et al.*, 1998). The RARβ gene can also be an integration site of HBV (de The *et al.*, 1987). It has been found that a malfunction of RXRα due to aberrant phosphorylation is associated with the development of HCC. This finding suggests that the loss of retinoid receptors, especially RXRα, plays a critical role in chemically-induced liver carcinogenesis in rats. Accordingly, acyclic retinoid supplementation can inhibit the phosphorylation of RXRα (Matsushima-Nishiwaki *et al.*, 2003) and increase the levels of RARα (Shimizu *et al.*, 2004), with a significantly reduced incidence of experimental HCC (Muto and Moriwaki, 1984; Kagawa *et al.*, 2004; Sano *et al.*, 2005). These observations suggest the restoration of retinoid receptors' function and expression. In particular, RXRα may be a potentially effective strategy for HCC prevention.

About 10–15% of patients with differentiated papillary and follicular *thyroid tumours* develop an aggressive disease with local recurrence and distant metastases as a consequence of their progressive inability to take up radioiodine (Arturi *et al.*, 1998). During the dedifferentiation process, the tumour progressively reduces the expression of the sodium iodide symporter (NIS), thyroglobulin (Tg) and thyroid-stimulating hormone receptor (Elisei *et al.*, 1994). Because *in vitro* studies have suggested that RA increases NIS mRNA expression and iodide uptake in some thyroid cancer cell lines (Van Herle *et al.*, 1990; Schmutzler *et al.*, 1997), RA treatment is under evaluation for its potential benefit in thyroid cancer therapy (Simon *et al.*, 1998; Gruning *et al.*, 2003). The anti-proliferative effect of RA in thyroid cancer cell lines may also rely on the ability of RA to decrease the secretion of VEGF, a major regulator of angiogenesis, in the thyroid gland (Hoffmann *et al.*, 2007). This effect correlates with the *in vivo* ability of RA to decrease tumour vascular surface density and

growth of xenotransplanted tumours (Hoffmann *et al.*, 2007). Thus, anti-angiogenic properties of RA may participate in RA-mediated anti-tumour activity.

Inhibition of vascular cell proliferation by RA has also been documented in *bladder cancer* (Hameed and el-Metwally, 2008). EGF and VEGF are up-regulated in superficial tumours and invasive tumours, and EGFR signalling and angiogenesis have been independently evaluated as targets for therapy. Natural and synthetic retinoids are known to reduce TGFα expression and EGFR expression and activation in experimental and human cancer models (Beenken *et al.*, 1999; Lango *et al.*, 2003). In bladder tumourigenesis, EGFR is an intermediate endpoint biomarker for retinoid chemoprevention (Hemstreet *et al.*, 1992). In *in vitro* studies, ATRA has been shown to reduce growth of both non-metastatic and metastatic bladder cell lines by antagonizing EGF-induced growth promotion (Nutting and Chowaniec, 1992). The association of RA with ketoconazole, which inhibits the P450 enzyme CYP26A thus preventing RA degradation (Rigas *et al.*, 1993; Lotan and Lotan, 2008), decreases the recurrence and prolongs the survival in patients suffering from bladder cancer. This effect seems to be related to the inhibitory effects of RA on both VEGF and EGF signalling (Hameed and el-Metwally, 2008).

Neuroblastoma is the most common extracranial solid tumour of childhood, and at least 40% of all children with neuroblastoma are designated as high-risk patients (Park *et al.*, 2008). Current treatment for high-risk neuroblastoma consists of a coordinated sequence of chemotherapy, surgery and radiation (Matthay *et al.*, 1999; Pearson *et al.*, 2008). Administration of the differentiating agent 13-*cis*-RA has been introduced in the therapy designed to eradicate minimal residual disease. 13-*cis*-RA exerts growth inhibitory and differentiating effects through its interaction with nuclear retinoid receptors, which regulate the expression of multiple target genes. When co-administered at an adequate concentration for up to 1 year after surgery, this agent has demonstrated to have some benefits in preventing complications in patients with neuroblastoma (Wagner and Danks, 2009).

Glioblastoma is the deadliest and most prevalent brain tumour. There is no effective therapeutic strategy for its treatment, and innovative approaches for the management of this deadly disease are greatly needed. Anti-invasive treatments may potentially limit the ability of glioma cells to infiltrate the surrounding brain tissue (Germanov *et al.*, 2006). In this regard, *in vitro* studies have suggested that RA in combination with the RXR ligand 6-OH-11-O-hydroxyphenantrene (IIF) may significantly affect growth, apoptosis, migration and invasive potential in glioblastoma U87MG cells (Orlandi *et al.*, 2003; Papi *et al.*, 2009). Moreover, the beneficial effects of the RXR agonist IIF may be enhanced by a combination treatment with the PPARγ agonist pioglitazone (PGZ). Promising effects have been obtained with the combined treatment of IIF and PGZ; it has been shown to reduce the migration, invasion, proliferation and viability of glioma cells from mice, rats and humans. Given the availability of these drugs, the present findings may be important for ongoing translational studies analysing the potential beneficial effects of PPARγ and RXR ligands in human clinical studies on glioblastoma patients.

Retinoid dual activity

Retinoids are used to treat of a wide range of diseases. Primarily, they are used for the treatment of a number of dermatological diseases including inflammatory skin disorders, psoriasis, photoaging and acne (Orfanos *et al.*, 1997). As mentioned earlier, in the last two decades, retinoids have been credited to have immunomodulation and anticancer properties. Each retinoid has a particular physiological and pharmacological effect, but some of them have both properties.

Among the first generation of retinoids, two molecules are worth mentioning: ATRA and 13-cRA. At present, ATRA is used to treat a number of haematological malignancies as it causes haematopoietic cells to differentiate and prolongs the lifespan of cells (He *et al.*, 1998; Orlandi *et al.*, 2003). Thanks to its immunological effect, ATRA has also been applied in Kaposi's sarcoma (Gill *et al.*, 1994). 13-cRA is a metabolic derivate of retinol and is present physiologically in small amounts in the body. It has been observed to be a potent modulator of inflammatory markers (Heliovaara *et al.*, 2007). Due to its immunomodulatory activity, especially stimulation of killer T cells, 13-cRA has been proposed as a cause of inflammatory bowel disease (Reddy *et al.*, 2006). On the other hand, 13-cRA has been used as a chemopreventive tool in head and neck squamous cell carcinoma (Klaassen *et al.*, 2001).

As a second generation retinoid compound, 9-cRA shows anticancer and immunomodulatory properties. It is indicated as a topical retinoid in AIDS-related Kaposi's sarcoma and experimental evidence suggests that it could be used for chemoprevention in breast, prostate and liver cancers (McCormick *et al.*, 1999; Teplitzky *et al.*, 2001; Tatebe *et al.*, 2008). In addition, 9-cRA shows an immunosuppressive effect on human monocyte differentiation in immature dendritic cells (DCs), as well as an inhibitory effect on inflammatory responses of microglia and astrocytes (Xu and Drew, 2006; Zapata-Gonzalez *et al.*, 2007).

There is also a third generation of retinoid compounds. Bexarotene, which only binds specifically to RXRs, is indicated as a treatment for T cell lymphoma. Case reports suggest bexarotene has a qualitative effect on CD8+ T cells as well as on CD8 T cell number (Berg *et al.*, 2008; Kamstrup and Gniadecki, 2008).

These findings suggest that the immunomodulatory and anti-cancer properties of retinoids are closely intertwined.

Conclusions

Retinoids are vitamin A derivatives that critically regulate several physiological and pathological processes, including immune functions and cancer development. These biological response modifiers exert their pleiotropic effects through their interaction with nuclear receptors, defined as RA receptors (RARs) and retinoid X receptors (RXRs). RARs and RXRs are also capable of interacting with other nuclear receptors, thus expanding their spectrum of action on gene expression. Evidence has been accumulated indicating that retinoids may exert beneficial effects in both immune-mediated disorders and tumours. With regard to cancer, retinoids directly target

neoplastic cells by inducing differentiation, inhibiting cell growth or promoting survival. However, the efficacy of these compounds in cancer treatment probably also resides in their ability to modulate the function of immune effectors. Vitamin A derivatives are currently used in the therapy of APL and cutaneous T cell lymphomas, but they could also be effective on B cell malignancies. Clinical trials are ongoing to test their efficacy in solid tumours (Montrone *et al*., 2009).

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Conflicts of interest

No conflicts of interest were declared

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