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Retinoids and retinoic acid receptor in cancer

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Abstract

Retinoids are vitamin A derivatives that exert major effects on growth control, epithelial differentiation and embryonic development and, accordingly, are highly teratogenic. In HL60 myelo-monocytic or F9 embryonal carcinoma cell lines, retinoic acid (RA) turns a transformed cell line into terminally differentiated non-proliferating cells. It was only in the mid 1980s that the cloning of the retinoic acid receptors gave a molecular basis to the pleiotropic action of these compound. Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are nuclear receptors, like those for steroids or thyroid hormones. They are hormone-activated transcription factors that bind to the promoters of their target genes as RAR/RXR heterodimers and repress or activate their transcription depending on the presence of the hormone. Both retinoic acid and its three RAR (α, β, γ) receptors have close links to cancer. Retinoids have significant antitumour activities in several clinical settings, in particular in cervical, skin, head and neck cancers, neuroblastoma and oral leukoplakia, where high doses have yielded significant improvements. A better understanding of the function targeted in the cancer cell and the receptor involved may lead to more specific drugs. Alterations of RARβ expression were reported in a variety of tumors and have suggested that it may act as a tumour suppressor. The clearest illustration of how alterations in RAR structure are linked to cancer is acute promyelocytic leukemia (APL), where a chromosome translocation fuses the PML gene to that of RARα, creating a PML/RARα fusion protein. Strikingly, in contrast, the previous malignancies that are RA-responsive, APL cells undergo terminal differentiation *ex vivo* or *in vivo*, similar to HL60 or F9 cells. APL therefore became the paradigm for differentiation therapy. Remarkably, APL is also exquisitely sensitive to arsenic trioxide and both RA and arsenic directly target the PML/RARα fusion protein, identifying the first example of oncogene-targeted therapies. Animal models have helped model the effects of these drugs and provide invaluable preclinical systems for one of the best understood malignancies to date.

Keywords: Receptors; Vitamin A; Leukemia; Arsenic; Transformation

1. Introduction

Only a few nuclear receptor ligands seem to play such an important role throughout life, from the earliest days of embryogenesis, to the function of terminally differentiated organs as complex as the brain, as retinoids do. Not unexpectedly, taking this importance, retinoids have been directly or indirectly implicated in many aspects of human cancer. A flurry of reviews have addressed the issues of retinoid biosynthesis, nuclear receptor function and links to cancer [1]. We will summarise then with a special emphasis on the disease which is the most intricately associated to retinoids, both in its pathogenesis and its therapy, acute promyelocytic leukaemia (APL).

2. Retinoids

Retinoids represent a group of natural or synthetic derivatives that activate the retinoic acid receptors pathways. Natural retinoids are derived from vitamin A or retinol. The latter comes either preformed or as it provitamin β carotene, in a variety of food such as milk, eggs and many plants. After its uptake by the intestinal mucosae, retinol becomes esterified and reaches the liver through the blood chylomicrons. The principal storage organ for retinol is the liver. Mobilisation ensues cleavage of the ester bond and retinol is transported in the plasma tightly bound to a specific transporter, RBP.

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Retinol then enters the cells where it is bound by specific proteins CRBP I and II, and later oxydised into retinal and retinoic acid (RA). The enzyme responsible for oxydisation is retinaldehyde deshydrogenase 2, as recently shown from knock-out studies. CRABP I and II also bind RA, which is then degraded in a P450-dependent manner. Remarkably, most of the players in RA metabolism are themselves RA-activated genes, implying a very complex feedback system from the very start of the biosynthesis pathway.

In fact, the complexity of retinoid metabolism is such that the role of some naturally occurring derivatives is unclear. This is particularly the case for 9-*cis* RA, an isomer of RA that has the remarkable ability to simultaneously bind both classes of receptors retinoic acid receptors (RARs) and retinoids receptors (RXRs) (see below). A variety of other natural ligands for the receptors or of potent retinol derviatives have also been described, but how exactly they function is presently unclear. The key point is that, in contrast to many other hormones, in particular hormones for nuclear receptors, retinoid production is a local one, directly in the target cell or tissue. No storage of preformed hormones was described, stressing the role of the enzymes implicated in either RA formation or degradation. An open question is to understand how these are regulated, as it underlies part of the proposed morphogenic action of these compounds. A flurry of synthetic retinoids have also been obtained. They have pro- or antiretinoid activities. The most interesting ones are specific for one receptor subtype and have been used in experiments aimed at implicating these specific receptors in defined biological effects. To date, there are few data derived from clinical trials using these agents (see below).

3. RARS

From the turn of the century to the mid-1980s, the retinoid field was an orphan one because, whereas very potent effects had been observed, notably in embryogenesis, organogenesis or cell transformation, their mode of action was not at all understood. The cloning of nuclear receptors that bound and were activated by RA completely changed the field. Moreover, the fact that one of the receptors was identified as the likely initiating event of a cancer underlined from the start their potential role in transformation [2].

No less than six retinoid receptors have been described. They fall into two distinct families, the RARs and the RXRs. As nuclear hormone receptors, they bind DNA, on specific elements known as retinoic acid responsive elements (RARE) that consist of a AGGTCA consensus directly repeated with a 5-bp spacing [3]. The receptors bind as a dimers and activate or repress transcription of target genes containing a RARE in a ligand-modulated manner. The functional DNA binding complex responsible for retinoid signalling appears to be the RAR/RXR heterodimer. In transient transfection assays, RXR homodimers may also bind DNA and activate transcription, but the *in vivo* physiological significance of these findings is still unclear. When bound to DNA, the RAR/RXR heterodimer bind corepressor molecules such as N-COR or SMRT. These large molecules recruit a protein complex with histone deacetylase activities which induces a compacted state of chromatin and hence repress transcription (Fig. 1, top left). Thus, in their unliganded state, the heterodimer represses its target genes. Upon ligand activation, binding of corepressors is destabilised allowing the sequential

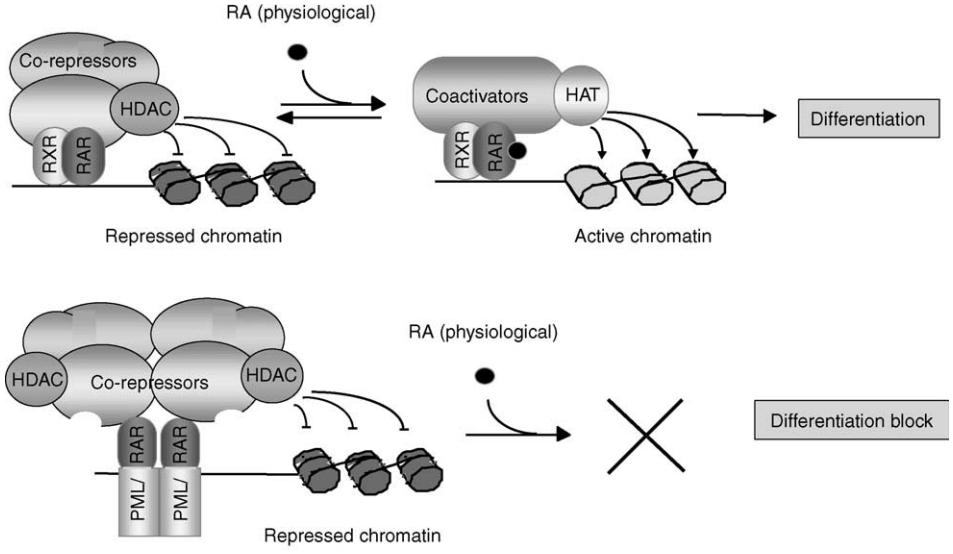

Fig. 1. Mode of action of retinoic acid receptors and transcriptional repression by PML/RARα.

binding of coactivator molecules of the p160 family, which recruits a complex with histone acetyl transferase activity that opens up the chromatin, and the DRIP complex, which recruits the basal transcription machinery, including RNA Pol II and directly induces target gene activation (Fig. 1, top right).

A complex set of gene knock-out experiments has shown that these different receptors have some specific functions, although there are significant overlaps. The situation is made much more complex by the fact that RXRs are promiscuous heterodimerisation partners for type II nuclear receptors. Therefore, ligands binding RXR molecules (rexinoids) have the potential to modulate other signalling pathways. In addition, through ill-defined mechanisms, retinoids exert major effects on other pathways, the best characterised being AP1, a serum-responsive transcriptional activator that plays an important role in inflammation and tumour progression. Thus, retinoids have the ability to modulate expression of genes that are not primary targets (that do not contain a RARE). Whereas the molecular details are still very unclear, dissociated retinoids have been generated that either exclusively activate target genes, or exclusively downregulate AP1 activity [4].

4. RARs in cancer

Association between retinoids and cancer were noted as early as the 1970s when retinoic acid treatment of a number of cell lines abolished their anchorage-independent growth, a property tightly associated to malignant transformation and to AP1 activation. Similarly, in the skin of mice topically treated with DMBA and TPA, retinoic acid treatment abolished the TPA-triggered promotion step. Later, retinoic acid triggered differentiation of HL60 cells and F9 cells yielded the first widely studied model of hormone-induced differentiation of a malignant cell towards a normal one. These observations prepared the concept of differentiation therapy that later exploded with acute promyelocytic leukaemia (APL) (see below).

Links between RARs and cancer are underlined by the fact that the RARs were discovered by the analysis of a HBV virus insertion site in a hepatocellular carcinoma [2]. The virus had inserted within the exon of a gene later identified as RARβ and created a fusion transcript which is believed to have acted in a dominant negative manner. Later, the constant involvement of RARα in acute promyelocytic leukemia provided an even more dramatic illustration of how an altered RARα can directly yield transformation (see below). A number of alterations in the expression of RARβ have been noted in a variety of tumours, particularly in the upper aerodigestive track and lung, suggesting that PAPβ may act as a tumour suppressor. Interestingly, RARβ constitutively represses AP1, which might be the basis for its recurrent loss in these clinical settings. Even in the absence of detectable molecular abnormalities in the RA transduction pathways, administration of retinoids has a favourable impact on many tumour cells [1]. Apart from APL (see below), retinoid administration is clearly beneficial in the following oral leukoplakia, undifferentiated thyroid cancers, neuroblastomas, cervical cancers and prevention of relapse in head and neck cancers. It is likely that with a better understanding of signalling pathways in transformed cells, and as better drugs come to the clinic, these indications are likely to extend.

5. Retinoic acid and APL

To date, the most striking example of retinoid efficiency is the case of APL. Remarkably, APL combines a specific alteration of RARα to an exquisite clinical sensitivity to the drug that is commonly used in clinical practice [5]. APL is induced by a t(15,17) translocation that generates a PML/RARa fusion protein [6]. Accordingly, PML/RARα expression induces leukemia in mice [7]. APL is a model disease for cancer biology, because it responds clinically to RA, which induces *in vivo* differentiation of the leukemic clone [8,9]. This differentiation, that is sometimes accompanied by the rapid proliferation of myeloid cells known as the RA-syndrome, yields clinically complete remissions. These are unfortunately short-lived if chemotherapy is not added. Combining RA treatment to chemotherapy has radically changed the prognosis of this malignancy, which

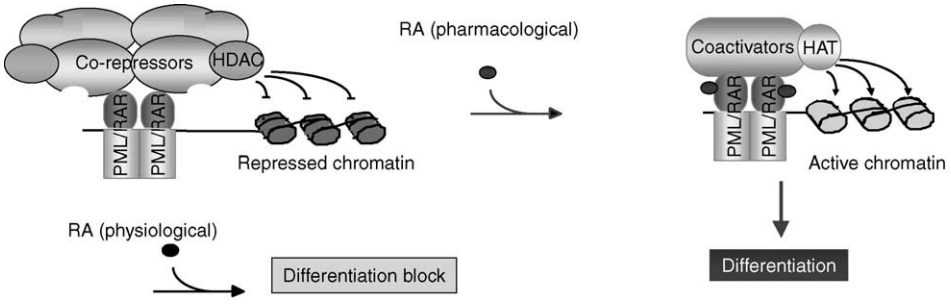

Fig. 2. Effect of RA to differentiate APL cells.

now has the best prognosis among myeloid leukaemias [9]. Since RA directly targets the PML/RARα oncoprotein, RA treatment of APL represents the first example, not only of differentiation therapy, but also of oncogene-targeted treatments [10,11].

A number of studies have addressed the molecular basis of RA response. Briefly, it is proposed that PML/RARα binds corepressor molecules more tightly than RAR, either because of an additional corepressor binding site (as in the rare, RA-resistant APLs associated to t(11,17) translocations and PLZF/RARα fusions) or because PML/RARα forms homodimers that are more efficiently associated to corepressors [12] (Fig. 1, bottom). Hence, like several other oncogenic fusion proteins associated to myeloid leukaemias, APL would be a disease of super-repression. The effect of pharmacological RA concentrations is then to dissociate the corepressors and induce activation through the chimera, which would then become a transcriptional activator [13,14] Fig. 2. A number of different laboratories have also shown that RA induces the degradation of the PML/RARα protein (see below) [15–17].

In a second step of analysis, it was realised that there was likely to be a reason underlying the fact that RARα was fused to PML in the overwhelming majority of the cases. Indeed, if the above mechanism accounted for all aspects of APL pathogenesis, why would PML be the (almost) unique fusion partner, rather than any protein containing a dimerisation interface and/or a corepressor binding site? It became progressively apparent that PML has growth suppressive properties [18]. Indeed, its overexpression was shown to block growth, to induce apoptosis or growth arrest [19,20]. Moreover, systematic alterations of PML expression were observed in a variety of human tumours [20,21]. However, it was the realisation that PML-null animals are highly sensitive to a variety of carcinogens and that PML-null cells are resistant to a variety of apoptogenic signals that gave a strong support to these initial observations [22,23]. PML has also attracted a lot of attention from cell biologists because of its association to discrete nuclear subdomains known as PML nuclear bodies [24–26]. These are extrachromosomal nuclear matrix-associated protein aggregates [27]. They are organised by PML following its sumolation and are composed of a large number of proteins, including key regulators of cell growth or transformation such as P53, Rb and Daxx [28–30]. Their function is ill understood, but they seem to play a role in senescence or growth control, at least in part by regulating the free amount of P53 or Daxx, although a more general role in the recruitment of missfolded proteins and possible catabolism was also recently proposed.

Arsenic trioxide was later shown to induce remissions in APL patients, essentially through induction of differentiation, although some apoptosis also exists *in vivo* [31–34]. Strikingly, arsenic also targets the PML/RARa protein, inducing its degradation through the specific induction of PML sumolation [29,35]. Normal PML proteins were also degraded, after being targeted to the nuclear matrix on PML nuclear bodies. Although degradation of the PML/RARα oncogene could well account for remission-induction, it may also be a secondary phenomenon (see below). The pathways involved in both RA- and As_2O_3- triggered PML/RARα degradation have been recently reviewed [11]. Briefly, RA induces the activation of the transactivation domain AF2 from RARα, which was shown to bind the SUG1 component of the 19S proteasome complex [36]. Hence, as for a variety of other transcription factors, activation is tightly coupled to catabolism. Similarly, As_2O_3 induces a rapid sumolation of either PML or PML/RARα and recruits the 11S complex of the proteasome, associated with their degradation.

These initial studies had outlined a similarity between the molecular effects of RA and As_2O_3, which was apparently difficult to reconcile with the fact that one induced differentiation while the other induced apoptosis. Yet, it progressively appeared that the clinical effects of the two drugs may be quite similar, in particular that both principally trigger differentiation, with some associated apoptosis in the case of As_2O_3 [34]. Later studies confirmed the predominance of differentiation in a variety of settings: use of low doses *ex vivo*, animal models, combination with cytokines or cAMP [34,37,38]. A clearer picture now emerges in which As_2O_3-induced changes in APL cells *in vivo* are associated to differentiation and hence are quite similar, if not identical, to those triggered by RA. It is conceivable that, in addition, As_2O_3 promotes some apoptosis. It should be stressed that induction of apoptosis is not necessarily a direct positive effect of As_2O_3, but could also occur by neglect. Indeed, in APL, RA triggers both strong pro- and anti-apoptotic signals (such as NFkB and Mcl1) [39]. Arsenic, in contrast, does not trigger these anti-apoptotic pathways. Therefore, apoptosis induction may result only from the fact that As_2O_3 triggers part of the genetic program associated with PML/RARα modulation only. Moreover, several groups have demonstrated a sharp *in vivo* synergy between the two agents in mice [34,40,41] and, more recently, in APL patients. If the clinical course of the two treatments is so similar, it is tempting to speculate that the molecular mechanisms of action of these two drugs are similar.

The molecular models that have been put forward to account for the efficacy of RA or As_2O_3 *in vivo* must take in account those *in vivo* therapeutic findings. The classical model accounts for many of the findings made so far, including enhancement of RA action by TSA and insensitivity of t(11,17) APL. The clinical efficacy of As_2O_3 and its enhancement of RA-induced differentiation

somehow question this model. Indeed, should PML/RARa actively induce differentiation through a *direct* RA-triggered transactivation of target genes, it seems paradoxical that As_2O_3-induced PML/RARα degradation *enhances* clinical response to RA. This in turn would favour a model in which PML/RARα (or other RARα fusions associated to APL) represses key myeloid differentiation genes, inducing the APL differentiation block. Effective therapies should then overcome this repression by releasing PML/RARa tethered corepressor complexes. This can be achieved through RA-triggered release or PML/RARa degradation. Another intriguing possibility was recently raised by the suggestion that, through an activation of the JNK pathway, As_2O_3 may destabilise the RARα/corepressor binding, again resulting in derepression of target genes [42]. Yet, targeting of the corepressor/nuclear receptor dimerisation interface would be difficult to reconcile with the specificity of arsenic action towards APL cells.

Whatever the mechanism, it appears that both RA and As_2O_3 directly target the PML/RARa complex responsible for disease induction and therefore represent the first example of oncogene-targeted therapy [10,11], long before the much publicised GLIVEC, in Europe STI 571. We have recently obtained direct evidence for the fact that both RA and As_2O_3 directly target PML/RARα by RNA arrays, which have demonstrated that many of the primary target genes of RA are also activated by As_2O_3. The clear demonstration that PML/RARα is a pharmacological target, whose manipulation suffices to induce remissions or cures, could help define drugs with enhanced specificity and even fewer side-effects.

The power of mouse genetics combined to the availability of oncogene-targeted therapies has led to the testing of the efficiency of a variety of drugs in these animals [43]. Several transgenics expressing PML/RARα or PLZF/RARα were challenged with RA, As_2O_3, histone deasactylase inhibitors (TSA, butyrate...). PML/RARα transgenics are exquisitely sensitive to both RA and As_2O_3, while PLZF/RARa transgenics are partially sensitive to RA and resistant to As_2O_3 [34,40]. Similarly again, transgenics harbouring a point mutation in the RA-binding domain are resistant to RA [44]. Similarly, RA effects are enhanced by histone desacetylase inhibitors, which converts RA-resistant PLZF/RARα into RA-sensitivity [45]. Hence, this first paradigm for oncogene-targeted cancer therapies also yielded the first example of therapy modelling in animal models of the disease.

6. Conclusion

To date, the most visible aspect of retinoid therapy of cancer is APL. Yet, in other diseases (neuroblastomas, leukoplakia, head and neck cancers...), retinoids have become part of the therapeutic strategies. It is most likely that, in the near future, a better understanding of the critical cross-talks or better drugs targeted at defined receptors may extend the therapeutic revolution of APL to other diseases. It is also important to note that 'atypical' retinoids may lead to some surprises. These compounds bind RARs, yet their very potent effects to induce apoptosis probably result from interference with other signalling pathways. Finally, retinoids may reveal their properties in combinations with other potent agents, such as interferons or histone deacetylase inhibitors, that share significant properties with RA, including induction of differentiation and growth arrest. While the APL story came to light largely by chance, it is likely that the coming progress in retinoid therapies of cancer will be the result of the basis science that has been accumulated in the past 15 years.

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