Drugs of the Future 1998, 23(4): 415-421

CCC: 0377-8282/98

Review Article

Retinoids in differentiation and cancer research

Luigi M. De Luca

Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institute of Health, Building 37, Room 3A-17, Bethesda, MD 20892-4255, USA

CONTENTS

Summary 415
Introduction 415
Epithelial function of retinoids 416
Retinoid receptors 417
Retinoids in cancer 418
Chemoprevention of epithelial cancer 418
Differentiation therapy 419
The molecular defect in acute promyelocytic leukemia 419
Synthetic retinoids 419
Cellular retinoic acid binding proteins 419
Conclusions 420
References 420

Summary

Nutritional research has highlighted the fundamental importance of vitamin A and its derivatives (the retinoids) in vision, reproduction, differentiation and growth, and has paved the way to the appreciation of the importance of these compounds in the prevention and possible therapy of diseases that involve the lining epithelia of the body, including cancer. In fact, retinoids are able to inhibit or reverse the formation of the preneoplastic lesion squamous metaplasia and to inhibit epithelial tumorigenesis in various animal models. Molecular biological approaches have permitted the discovery, nearly a decade ago, of the two families of retinoid receptors, the RARs and the RXRs, with all-*trans*-retinoic acid (RA) and 9-*cis*-RA as their respective ligands. The RXR family of receptors mediates the action of several other hormone receptors, including the RA receptor, vitamin D receptor and thyroid hormone receptor, with which they form heterodimer RXR-RAR, RXR-vitDR and RXR-TR. RAR and RXR transcript (mRNA) expression has been shown to be cell type specific: RAR-β transcripts are abundant in mucus-secreting cells and absent from epidermal keratinocytes, RARγ transcript expression is abundant in keratinocytes and absent from mucous cells and RARα expression is ubiquitous.

Skin tumorigenesis causes the selection of cells with reduced expression of RARs, whereas RXR expression does not seem to be affected in the malignant tumors. Mesenchymal tissue tumors are also affected by RA. Acute promyelocytic leukemia (APL) is characterized by a balanced chromosomal translocation (t:15;17) with breakpoints in the A/B region of the RARα gene on the long arm of chromosome 17 and the PML gene on the long arm of chromosome 15. RA induces complete remission in most patients with APL, although resistant clones do eventually emerge, for which combined chemotherapy and differentiation therapy is being considered. In addition to the receptors, other retinoid binding proteins are important for retinoid action and appear to be influenced by the carcinogenesis process.

The search for synthetic compounds that might specifically activate one or more of the various retinoid receptor-mediated signal transduction pathways is of fundamental interest, since it may lead to treatment options for a variety of diseases.

Introduction

Remarkable progress has been made in the past decade in our understanding of the mode of action of vitamin A and its derivatives, the retinoids (1). This progress is due to the discovery of the nuclear receptors which bind the retinoids, thereby presiding over a large network of gene activation reactions (2). The specificity with which some of these receptors bind different retinoids makes them a finely tuned tool to guide gene activation processes.

Figure 1 shows the structure of the parent compound, retinol, and its oxidation product, retinoic acid (RA), in the stretched all-*trans* configuration at the top of the figure. The 9-*cis*-retinoic acid (Fig. 1) is the highest affinity ligand for the two families of nuclear receptors, the retinoic acid receptors, RARs (3), and the retinoid X receptors, the RXRs (4), so named before the chemical structure of its ligand, 9-*cis*-RA, was discovered (5). The specificity of interactions would also suggest the possibility that various retinoids, both natural and synthetic, may specifically be useful as drugs to combat diverse diseases.

Most of the work thus far has focused on dermatological and neoplastic diseases. However, the development of retinoid structures to address other diseases is also progressing.

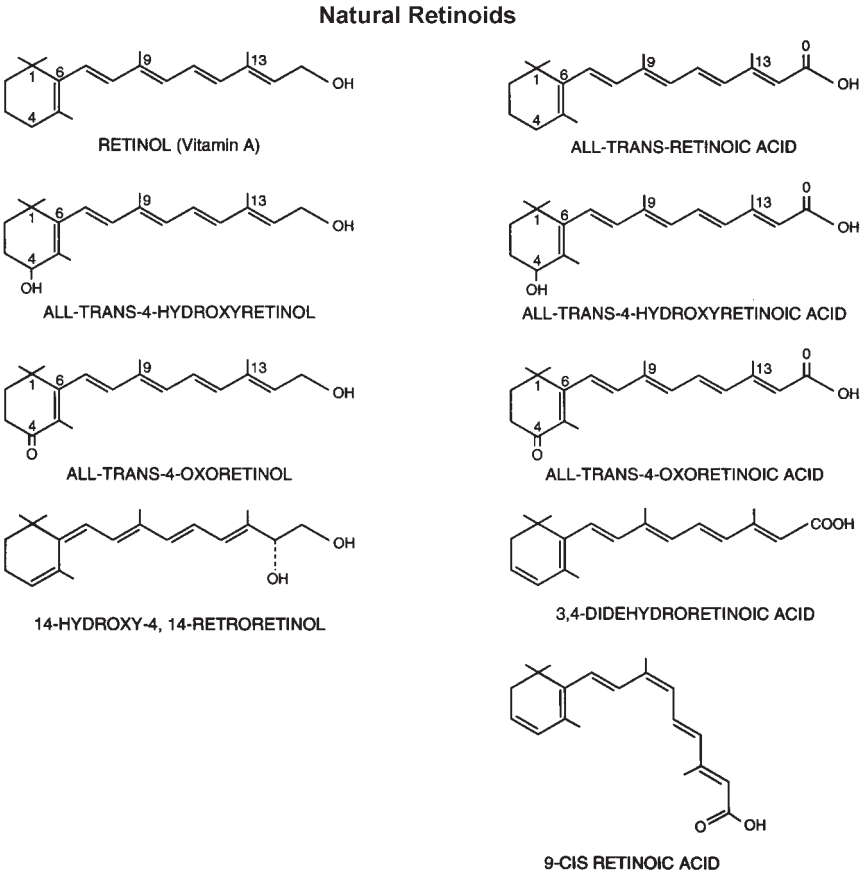

Fig. 1. The chemical structure of some of the most common natural retinoids.

Other structures shown in Figure 1 represent various oxidation products with partial biological activity and as yet undetermined biological function *in vivo*.

The reason for the use of retinoids in cancer as differentiation therapy agents is based on the background knowledge that epithelial differentiation is strictly dependent on vitamin A (6). In addition, retinol (vitamin A) is the only retinoid known to be capable of sustaining all vitamin A functions, including development, vision and reproduction (7, 8). RA, on the other end, maintains differentiation and growth (9) of the adult organism. It is then obvious that several retinoids work at some different level and in concert to warrant the health and well being of the entire organism.

Epithelial function of retinoids

In the adult, the most evident transformation resulting from vitamin A deficiency occurs in epithelial tissues, particularly in mucus-secreting epithelia. Profound changes take place whether the epithelium is of the simple-columnar type, *i.e.*, made up of a single row of columnar cells (Fig. 2) as found in the lining epithelium of the endocervix and uterine cavity, or the more complex pseudostratified epithelium of the trachea, made up of both columnar as well as basal cells, or the most complex type of mucus-secreting epithelium, the stratified-columnar epithelium (Fig. 2) found in the conjunctiva of the eye. The use of antibodies to the keratins K8 (specific for mucous cells) and K5 (specific for squamous cells) has permitted the definition of the various steps during the formation of the squamous-metaplastic focus. The onset of vitamin A deficiency is readily observed in simple-columnar epithelia (K8-positive and K5-negative), which become initially pseudostratified by the emergence of basal cells that express the keratin K5 (Fig. 2). As deficiency progresses, these basal cells divide and form into squamous-metaplastic foci, which eventually occupy the entirety of the basement membrane, thereby replacing the columnar cells (Fig. 2). This process affects all mucus-secreting epithelia of the body (except for the intestinal epithelium) and profoundly alters the functional characteristics of these tissues (10). The resulting phenotype of these lining epithelia, shown schematically at the bottom of Figure 2, is morphologically similar to vaginal epithelium during estrous and to epidermis. The squamous-metaplastic epithelium ceases to express RARβ and acquires the expression of RARgamma (Fig. 2). In the intact animal this transformation is not compatible with survival, mainly because infectious agents, normally disposed of by

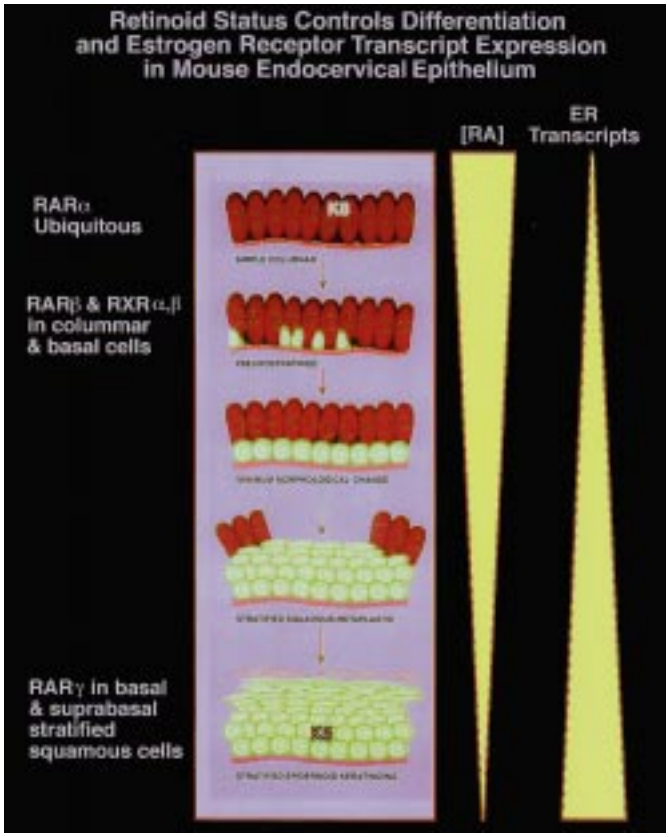

Fig. 2. The stepwise process of squamous metaplasia due to retinoid depletion. Shown is the stepwise pathological process of squamous metaplasia formation in the simple-columnar epithelium of the endocervix. A single row of RARbeta and keratin K8-positive columnar cells characterizes the simple-columnar epithelium of the endocervix, as it is under normal conditions of vitamin A nutriture. Under conditions of vitamin A deficiency, RARgamma and keratin K5-positive basal cells appear in subcolumnar positions. Eventually a continuous layer of subcolumnar basal cells lifts the columnar cells, which cease to divide and are shed off, while the K5-positive cells proliferate and form a stratified squamous-metaplastic focus. Different foci merge into a stratified squamous keratinizing epithelium. These changes are reversible upon administration of vitamin A. The figure also indicates changes in the expression of retinoid and estrogen receptor transcripts during the establishment of squamous metaplasia.

mucociliary activity, adhere to the metaplastic epithelium and eventually cause infectious diseases.

In populations suffering from vitamin A deficiency, mortality due to measles and other infectious diseases is high and can be alleviated by administration of the vitamin (11, 12). Recent work has also shown that administration of vitamin A prevents the transplacental transfer of HIV infection from mother to offspring (13, 14). While nutritional supplementation of vitamin A in populations at high risk for infectious diseases are important intervention strategies, this is just the beginning of retinoid application to disease prevention. In this approach, natural as well as synthetic retinoids will be used as specific drugs.

Retinoid receptors

There are three RAR genes (RARα, β, γ) and three RXR genes (RXRα, β, γ) and multiple isoforms for each of the receptors, resulting from either alternative RNA splicing or the use of different promoters (15). This would suggest a multiplicity of functions or functional sites, or both, for these receptors.

We probed cell type specificity of RAR and RXR expression by *in situ* hybridization analysis. This work has indicated that columnar cells of the endocervical and uterine epithelia express in prevalence RARβ transcripts (Fig. 2) (10, 16); in sharp contrast, the squamous keratinizing epithelium of the epidermis and vagina express high levels of RARγ and little RARβ (17). RARα shows ubiquitous transcript expression in all cell types (Fig. 2).

The process of carcinogenesis greatly downregulates RAR expression, without significant effects on RXR transcripts (17). These transcripts appear high in proliferating cells of the epidermis and their tumors.

The distinctive characteristic of RXRs is their ability to interact with other nuclear receptors of the superfamily and to bring them to interact with their respective DNA response elements in the promoters of different target genes (18). Gene transcription responses are RXR partner receptor-dependent: for example, they are vitamin D_3-dependent if the RXR partner receptor is the vitamin D

receptor (RXR-vitDR heterodimer), thyroid hormone-dependent for the RXR thyroid hormone receptor partner (RXR-TR heterodimer), and so on. It is, therefore, easy to conceptualize how RXRs may control a complex network of hormone-dependent pathways. This complexity would explain the far reaching consequences of vitamin A deficiency, a condition which is indeed incompatible with the life process itself from conception through embryonic development to maintenance of the adult organism.

The patterned expression or suppression of developmental genes (*e.g.*, the homeobox b-1 gene) has been demonstrated to be strictly retinoid responsive in specific rhombomeres of the mouse hindbrain (19). In fact, RA response elements (RAREs) have been demonstrated for both 5' and 3' of the gene with the 5'-RARE functioning as a suppressor and the 3'-RARE as an inducer of transcriptional activity for the homeobox b-1 gene (20). The developmental time dependency of the function of the two RAREs permits and controls the switching on and off of the homeobox b-1 gene expression and termination of expression at the border of different rhombomeres, possibly controlling segmentation. It is also evident that a highly ordered retinoid delivery system must be operative to insure the highly regulated series of developmental events. Availability of excess retinoids or their deficiency may lead to teratogenesis and/or resorption of the embryo (21-23).

We will not dwell on dermatological applications of retinoids because they have been used for over 15 years and are covered elsewhere in the literature. Suffice it to say that the most common application of retinoids has been in the therapy of dermatological conditions, particularly juvenile acne (24). Oral RA is very effective in reversing the pathology of juvenile acne, although its teratological effects are also well known (21, 22). Therefore, the use of topical retinoid therapy is now preferred.

Retinoids in cancer

What has been summarized above is intended to also explain the expectation that retinoids would be viewed as chemopreventive and differentiation therapeutic agents. Chemoprevention research emphasizes the concept that retinoids, when available optimally at supraphysiological levels, inhibit the development of epithelial carcinogenesis, *i.e.*, their action is exerted during the carcinogenesis process (25). The concept of differentiation therapy proposes the use of agents capable of eliminating neoplastic cells through differentiative (1) rather than cytotoxic pathways. Retinoids have been employed in both approaches.

Chemoprevention of epithelial cancer

In experimental animal models of epithelial carcinogenesis, retinoids have shown considerable activity as chemopreventive agents. The most widely used system, probably because of the ease of observation of the tumors and scoring of results, has been the system of mouse skin carcinogenesis. In this system, conditions have been defined so that the use of one application of the carcinogen 7,12-dimethyl benzanthracene (DMBA) is necessary but not sufficient for tumor induction, which is manifest only after multiple applications of the tumor-promoting agent 12-tetradecanoyl-phorbol-13 acetate (TPA) (26).

A stepwise process can then be observed with the formation first of benign tumors (papillomas) and eventually of malignant tumors (carcinomas). RA applied topically before the application of TPA greatly inhibits both papilloma and carcinoma formation (27). Interestingly, a specific inhibition of carcinoma formation is observed when RA is introduced in the diet at pharmacological concentrations (30 μg/g diet) (28).

The inhibitory action of RA on skin carcinogenesis appears to be exerted at the level of RARs. *In situ* hybridization analysis has shown that mouse epidermal neoplastic cells lose the ability to express RAR transcripts during tumor promotion and malignant progression. In particular, RARα transcripts are expressed in epidermis, papillomas and differentiated carcinomas, but are not expressed in undifferentiated carcinomas and in the most malignant spindle cell carcinomas. RARγ transcripts are abundant in epidermis and papillomas but are absent in both differentiated and undifferentiated carcinomas and in the more malignant spindle cell carcinomas. RARβ transcripts are totally absent in the epidermis. We also found that, in contrast to the observed reduction in RARα and RARγ during malignant progression, RXR transcript expression remains at a high level in proliferating cells of normal epidermis and tumor tissue (17, 29).

In vitro cell transformation studies of mouse epidermal keratinocytes by the oncogene *ras* demonstrated similar RAR transcript reduction with concomitant increased proliferation. RARγ gene expression in *ras*-transformed mouse keratinocytes has demonstrated RA-induced reduction in cell growth (29). Exposure of mouse skin to tumor promoters *in vivo* also demonstrated a reduction in receptor transcript expression (30). Precancerous lesions of buccal epithelium *in vivo* (31) and in cells from head and neck cancer (32) and non-small cell lung cancer patients (33) show a marked reduction in RARβ transcript expression (32). Therefore, it is suggested that the process of carcinogenesis selects for cells which have lost the ability to express RARs but maintain the ability to express RXRs, at least in the epidermal carcinogenesis model. Moreover, exposure of some of these cells to supraphysiological levels of RA may induce inhibition of cell growth through the induction of RAR gene expression. This and other relevant work makes the important point that loss of RAR function is an important contributory factor to loss of cell differentiation and the emergence of unregulated cell growth.

We suggest that the mechanism underlying the chemopreventive effects of supraphysiological RA is the enhanced expression of RARs, which counteracts

the downregulatory effects of tumor promoters. However, though logical, this mechanism remains to be demonstrated.

Differentiation therapy

A variety of neoplastically transformed cells have been shown to respond to RA by reduced cell growth and induction of differentiation. Among these cells, the human leukemia HL-60 cells were shown by Breitman and collaborators to undergo differentiation to mature granulocytes (34). This work inspired the use of RA in acute promyelocytic leukemia (APL). Remarkably, RA given systemically induced the disappearance of promyelocytes and the increase in mature granulocytes coincident with complete remission (35). RA administration for APL is now standard treatment in the clinic. This treatment is indeed successful in prolonging life in the majority of patients. However, RA resistance does occur through the eventual emergence of promyelocytic clones which fail to respond to RA (36, 37). In addition, RA effectiveness is undermined by self-induced metabolism, which causes a marked reduction in RA concentration in the blood of treated patients (38). New approaches to overcome this problem include combination treatments with RA and chemotherapeutic agents to eradicate the newly emerging RA-resistant clones (39).

The molecular defect in acute promyelocytic leukemia

Molecular biological approaches have permitted the definition of the molecular defect responsible for APL. In all cases, it appears that the APL clone contains a balanced (t:15;17) chromosomal translocation of the long arm of chromosome 17 to chromosome 15 and *vice versa* (40, 41). The breakpoint on chromosome 17 is the A/B region of the RARα gene which fuses with the breakpoint in the PML gene on chromosome 15. The prevailing chimeric protein found in blood cells of APL patients is PML-RARα, whereas the alternative variant, RARα-PML transcribed from the fusion gene on chromosome 17, is found only in small amounts in APL patients.

The mechanism responsible for the induction of complete remission by RA in patients with APL is unclear. It is not easy to rationalize why RA is effective in a disease in which one of its receptors is itself truncated and fused to PML (42-44). It is possible that RA may bind to RARβ and/or RARγ, which are not involved in the fusion with PML and therefore may still be functional and capable of inducing the differentiation to granulocytes, at least at pharmacological concentrations of RA.

Synthetic retinoids

The rationale for the development of synthetic retinoids is based on the fact that different RA receptors may bind different synthetic retinoids, thereby presiding over different biological processes; as such, they may also combat different diseases. Synthetic retinoids may also be more effective than RA itself, because they may be designed in such a way that they will not undergo metabolism and may be present for much longer half-lives in the blood of patients. Synthetic work has permitted the realization of retinoid structures with remarkable specificity as RXR and RAR ligands.

Research at Allergan (45) has resulted in the development of several RAR and RXR-selective retinoids. A comparison between RAR- and RXR-selective retinoids for transcription of the TGase II gene in HL-60 cells has shown that both could activate TGase II gene transcription. This is because the TGase II gene promoter contains both an RARE as well as an RXRE (46).

Scientists at Ligand Pharmaceutical have demonstrated that RXR agonists function as activators of the insulin response in diabetic rats (47) and that the synthetic RAR-selective retinoid ALRT1550 functions as an inhibitor of the growth of buccal carcinoma in nude mice (48).

The work at Roche (49) with sterically blocked retinoids of the 9-*cis*-RA isolog type and without the possibility of back-isomerization to the all-*trans* configuration has also clearly shown that these compounds are highly active in the induction of HL-60 cell differentiation and the consequent inhibition of cell growth. Since these constrained structures are an order of magnitude more potent than the parent compounds, this strongly suggests that either the natural 9-*cis*-RA undergoes faster metabolism or isomerization, or both.

Cellular retinoic acid binding proteins

In addition to RAR expression, the expression of cellular RA binding proteins CRABP I and II also mediates the biological activity and/or the availability of RA (50). It has recently been shown that estrogen receptor (ER)-positive breast cancer cell lines express high levels of CRABP II, in contrast to ER-negative cells that show very low expression (51). It is also of interest that human epidermis CRABP II contains RAREs in its promoter. Therefore, it is not surprising that this protein is upregulated by RA, but this upregulation is not observed in certain ER-negative human breast cancer cells.

The picture emerges that RARs, CRABP II and other RA responsive genes such as TGase II are somehow refractory to RA action in certain cancer cells.

In particular, we have investigated 15 different cell lines and have observed that in the resistant cells RA cannot be metabolized to the same extent as in RA-sensitive cells. So, it appears that at least five parameters are in correlation: RA-mediated inhibition of cell growth, and induction of RAR expression, CRABP II, TGase II and RA metabolism. The RA growth inhibited cells all metabolize RA at a fast rate compared to resistant cells, which tend to accumulate the intact drug (52).

We have also found that expression of a truncated RARα (RAR403), which has dominant negative activity over all RARs, slows down RA metabolism and renders the cells resistant to RA-induced inhibition of growth and induction of TGase II. These findings suggest the involvement of RARs in RA-induced inhibition of cell growth. They also suggest that the observed resistance to RA in some malignant cells (52) may well be the result of a blunted RAR-mediated signal transduction pathway in these cells (Isogai *et al*., submitted). In fact, it has been demonstrated that some of these cell lines, in particular the ER-negative MDA-231 and others, express very low levels of the RARα (53) and that introduction of estrogen receptor expression (54) reestablishes RA-induced growth inhibition in these cells.

Conclusions

This brief review of the retinoid field is intended to provide a perspective of how these compounds have come to occupy a prominent place in the field of chemoprevention of carcinogenesis and differentiation therapy.

Other areas of relevance for the employment of retinoids derive from very recent discoveries. For instance, the finding that in mice with noninsulin-dependent diabetes mellitus and obesity, RXR agonists can enhance responsiveness to insulin, makes these compounds of potential therapeutic interest in this disease (47). The recent reports of the activity of retinoids in reversing the typical lesions of emphysema in a rat animal model of the disease (55) open up the possibility that retinoids may be useful drugs for the therapy of emphysema in humans.

Finally, it should be emphasized that the discovery of the retinoid receptors was possible through seemingly unrelated molecular biological research on steroid hormone receptors, and provides a paradigm for how basic research can foster the development of useful drugs.

References

1. De Luca, L.M., Darwiche, N., Jones, C.S., Scita, G. *Retinoids in differentiation and neoplasia.* Sci Am Sci Med 1995, 2: 28-37.

2. De Luca, L.M. *Retinoids and their receptors in differentiation, embryogenesis and neoplasia.* FASEB J 1991, 5: 2924-33.

3. Mangelsdorf, D.J., Thummel, C., Beato, M. et al. *The nuclear receptor superfamily: The second decade.* Cell 1995, 83: 835-9.

4. Mangelsdorf, D.J., Borgmeyer, U., Heyman, R.A. et al. *Characterization of three RXR genes that mediate the action of 9-cis retinoic acid.* Genes Dev 1992, 6: 329-44.

5. Levin, A.A., Sturzenbecker, L.J., Kazmer, S. et al. *9-cis Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα.* Nature 1992, 355: 359-61.

6. Wolbach, S.B., Howe, P.R. *Tissue changes following deprivation of fat-soluble A-vitamin.* J Exp Med 1925, 42: 753-78.

7. Dairkee, S.H., Ljung, B.M., Smith, H., Hackett, A. *Immunolocalization of a human basal epithelium specific keratin in benign and malignant breast disease.* Breast Cancer Res Treat 1987, 10: 11-20.

8. Wald, G. *The molecular basis of visual excitation.* Nature 1968, 219: 800-7.

9. Dowling, J.E., Wald, G. *The role of vitamin A acid.* Vitam Horm 1960, 18: 515-41.

10. Darwiche, N., De Luca, L.M. *Retinoids in differentiation and prevention of malignant transformation.* In: Intraepithelial Neoplasia of the Female Lower Genital Tract. Jordan, J., Luesley, D., Richart, R. (Eds.). Churchill Livingstone: London 1995, 309-24.

11. Sommer, A., Tarwotjo, I., Djunaedi, E., West, K.P. Jr., Loeden, A.A. *Impact of vitamin A supplementation on childhood mortality: A randomized controlled community trial.* Lancet 1986, 1: 1169-73.

12. Sommer, A. *Large dose vitamin A to control vitamin A deficiency.* Int J Vitam Nutr Res 1989, Suppl. 30: 37-41.

13. Semba, R.D., Scott, A.L., Natadisastra, G. et al. *Depressed immune response to tetanus in children with vitamin A deficiency.* J Nutr 1992, 122: 101-7.

14. Semba, R.D., Ward, B.J., Griffin, D.E. et al. *Abnormal T-cell subset proportions in vitamin-A-deficient children.* Lancet 1993, 341: 5-8.

15. Zelent, A., Mendelsohn, C., Kastner, P. et al. *Differentially expressed isoforms of the mouse retinoic acid receptor beta are generated by usage of two promoters and alternative splicing.* EMBO J 1991, 10: 71-81.

16. Darwiche, N., Celli, G., De Luca, L.M. *Specificity of retinoid receptor gene expression in mouse cervical epithelia.* Endocrinology 1994, 134: 2018-25.

17. Darwiche, N., Celli, G., Tennenbaum, T., Glick, A.B., Yuspa, S.H., De Luca, L.M. *Mouse skin tumor progression results in differential expression of retinoic acid and retinoid X receptors.* Cancer Res 1995, 55: 2774-82.

18. Kliewer, S.A., Umesono, K., Evans, R., Mangelsdorf, D.J. *The retinoid X receptors: Modulators of multiple hormonal signaling pathways.* In: Vitamin A in Health and Disease. Blomhoff, R. (Ed.). Marcel Dekker: New York 1994, 239-55.

19. Studer, M., Popperl, H., Marshall, H., Kuroiwa, A., Krumlauf, R. *Role of a conserved retinoic acid response element in rhombomere restriction of Hoxb-1.* Science 1994, 265: 1728-32.

20. Marshall, H., Studer, M., Popperl, H. et al. *A conserved retinoic acid response element required for early expression of the homeobox gene Hoxb-1.* Nature 1994, 370: 567-71.

21. Lammer, E.J., Chen, D.T., Hoar, R.M. et al. *Retinoic acid embryopathy.* New Engl J Med 1985, 313: 837-41.

22. Oakley, G.P. Jr., Erickson, J.D. *Vitamin A and birth defects. Continuing caution is needed.* New Engl J Med 1995, 333: 1414-5.

23. Kochhar, D.M. *Cellular basis of congenital limb deformity induced in mice by vitamin A.* In: Morphogenesis and Malformation of the Limb. Bergsma, D., Lenz, D.W. (Eds.). Alan R. Liss: New York 1977, 111-54.

24. Peck, G.L. *Retinoids in clinical dermatology.* In: Progress in Diseases of the Skin. Fleischmajer, R. (Ed.). Grune and Stratten: New York 1981, 227-69.

25. Sporn, M.B., Newton, D.L. *Chemoprevention of cancer with retinoids.* Fed Proc 1979, 38: 2528-34.

26. Chen, L.C., De Luca, L.M. *Retinoids and skin cancer.* In: Skin Cancer: Mechanisms and Human Relevance. Mukhtar, H. (Ed.). CRC Press: Boca Raton 1995, 401-24.

27. Verma, A.K., Conrad, E.A., Boutwell, R.K. A*n overview of cutaneous carcinogenesis.* Cancer Res 1982, 42: 3519-25.

28. Chen, L.C., Kirchhof, S., De Luca, L.M. *Effect of excess dietary retinoic acid on skin papilloma and carcinoma formation induced by a complete carcinogenesis protocol in Sencar mice*. Cancer Lett 1994, 78: 63-7.

29. Darwiche, N., Scita, G., Jones, C. et al. *Loss of retinoic acid receptors in mouse skin and skin tumors is associated with activation of the ras^{Ha} oncogene and high risk for premalignant progression.* Cancer Res 1996, 56: 4942-59.

30. Kumar, R., Shoemaker, A.R., Verma, A.K. *Retinoic acid nuclear receptors and tumor promotion: Decreased expression of retinoic acid nuclear receptors by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate.* Carcinogenesis 1994, 15: 701-5.

31. Lotan, R., Xu, X.C., Lippman, S.M. et al. *Suppression of retinoic acid receptor-β in premalignant oral lesions and its up-regulation by isotretinoin.* New Engl J Med 1995, 332: 1405-10.

32. Xu, X.C., Ro, J.Y., Lee, J.S., Shin, D.M., Hong, W.K., Lotan, R. *Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues.* Cancer Res 1994, 54: 3580-7.

33. Xu, X.C., Sozzi, G., Lee, J.S. et al. *Suppression of retinoic acid receptor beta in non-small-cell lung cancer in vivo: Implications for lung cancer development.* J Natl Cancer Inst 1997, 89: 624-9.

34. Breitman, T.R. *Growth and differentiation of human myeloid leukemia cell line HL60.* Methods Enzymol 1990, 190: 118-30.

35. Castaigne, S., Chomienne, C., Daniel, M.T. et al. *All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results.* Blood 1990, 76: 1704-9.

36. Warrell, R.P. Jr. *Pathogenesis and management of acute promyelocytic leukemia.* Annu Rev Med 1996, 47: 555-65.

37. Delva, L., Cornic, M., Balitrand, N. et al. *Resistance to all-trans retinoic acid (ATRA) therapy in relapsing acute promyelocytic leukemia: Study of in vitro ATRA sensitivity and cellular retinoic acid binding protein levels in leukemic cells.* Blood 1993, 82: 2175-81.

38. Adamson, P.C., Bailey, J., Pluda, J. et al. *Pharmacokinetics of all-trans-retinoic acid administered on an intermittent schedule.* J Clin Oncol 1995, 13: 1238-41.

39. Cornic, M., Delva, L., Castaigne, S. et al. *In vitro all-trans retinoic acid (ATRA) sensitivity and cellular retinoic acid binding protein (CRABP) levels in relapse leukemic cells after remission induction by ATRA in acute promyelocytic leukemia.* Leukemia 1994, 8: 914-7.

40. de Thë, H., Chomienne, C., Lanotte, M., Degos, L., Dejean, A. *The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus.* Nature 1990, 347: 558-61.

41. de Thë, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L., and Dejean, A. *The PML-RARα fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR.* Cell 1991, 66: 675-84.

42. Fagioli, M., Grignani, F., Ferrucci, P.F. et al. *Effect of the acute promyelocytic leukemia PML/RARα protein on differentiation and survival of myeloid precursors.* Leukemia 1994, 8 (Suppl. 1): S7-11.

43. Grignani, F., Testa, U., Rogaia, D. et al. *Effects on differentiation by the promyelocytic leukemia PML/RARα protein depend on the fusion of the PML protein dimerization and RARα DNA binding domains*. EMBO J 1996, 15: 4949-58.

44. Raelson, J.V., Nervi, C., Rosenauer, A. et al. *The PML/RARα oncoprotein is a direct molecular target of retinoic acid in acute promyelocytic leukemia cells.* Blood 1996, 88: 2826-32.

45. Nagpal, S., Athanikar, J., Chandraratna, R.A. *Separation of transactivation and AP1 antagonism functions of retinoic acid receptor α.* J Biol Chem 1995, 270: 923-7.

46. Nagy, L., Saydak, M., Shipley, N. et al. *Identification and characterization of a versatile retinoid response element (retinoic acid receptor response element-retinoid X receptor response element) in the mouse tissue transglutaminase gene promoter.* J Biol Chem 1996, 271: 4355-65.

47. Mukherjee, R., Davies, P.J., Crombie, D.L. et al. *Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists.* Nature 1997, 386: 407-10.

48. Shalinsky, D.R., Bischoff, E.D., Lamph, W.W. et al. *A novel retinoic acid receptor-selective retinoid, ALRT1550, has potent antitumor activity against human oral squamous carcinoma xenografts in nude mice.* Cancer Res 1997, 57: 162-8.

49. Apfel, C.M., Kamber, M., Klaus, M., Mohr, P., Keidel, S., LeMotte, P.K. *Enhancement of HL-60 differentiation by a new class of retinoids with selective activity on retinoid X receptor.* J Biol Chem 1995, 270: 30765-72.

50. Ong, D.E., Newcomer, M.E., Chytil, F. *Cellular retinoid-binding proteins.* In: The Retinoids: Biology, Chemistry, and Medicine, 2nd Ed. Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.). Raven Press: New York 1994, 303-4.

51. Jing, Y., Waxman, S., Mira-López, R. *The cellular retinoic acid binding protein II is a positive regulator of retinoic acid signaling in breast cancer cells.* Cancer Res 1997, 57: 1668-72.

52. Takatsuka, J., Takahashi, N., De Luca, L.M. *Retinoic acid metabolism and inhibition of cell proliferation: An unexpected liaison.* Cancer Res 1996, 56: 675-8.

53. Sheikh, M.S., Shao, Z.M., Chen, J.C., Hussain, A., Jetten, A.M., Fontana, J.A. *Estrogen receptor-negative breast cancer cells transfected with the estrogen receptor exhibit increased RARα gene expression and sensitivity to growth inhibition by retinoic acid*. J Cell Biochem 1993, 53: 394-404.

54. Sheikh, M.S., Shao, Z.M., Li, X.S. et al. *Retinoid-resistant estrogen receptor-negative human breast carcinoma cells transfected with retinoic acid receptor-α acquire sensitivity to growth inhibition by retinoids.* J Biol Chem 1994, 269: 21440-7.

55. Massaro, G.D., Massaro, D. *Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats.* Nat Med 1997, 3: 675.