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Review

Detection of altered retinoic acid receptor expression in tissue sections using in situ hybridization

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Summary. Nuclear retinoid receptors mediate retinoid effects in controlling cell growth, differentiation, apoptosis, and carcinogenesis. Altered expression or activity of these receptors could abolish the retinoid signal transduction pathway and be associated with human carcinogenesis. *In situ* hybridization is a powerful tool for analyzing gene expression in formalin-fixed, paraffin-embedded tissue sections, especially for newly cloned genes or when no antibodies are available. Detection of altered retinoid receptor expression using *in situ* hybridization in premalignant and malignant tissues has provided important information about the roles of these receptors in cancer development and the response of these tissues to retinoid treatment. Among these receptors, altered expression of retinoic acid receptor-ß (RAR-ß) has been mostly detected in human cancers, including those of the head and neck, lung, esophagus, mammary gland, pancreas, and cervix. RAR-ß is thus currently used as a surrogate endpoint biomarker in different clinical prevention trials of various cancers.

Key words: Retinoid, Nuclear retinoid receptor, Human cancer, *In situ* hybridization

Introduction

Retinoids, which are structural and functional analogs of vitamin A, are known to regulate various essential biological processes, e.g., vision, reproduction, metabolism, hematopoiesis, bone development, and pattern formation during embryogenesis (Lotan, 1980; Sporn et al., 1994). Pharmacologically, retinoids have been recognized as important factors in modulating cell growth, differentiation, and apoptosis and suppressing carcinogenesis in a broad range of tissue types (Lotan, 1980; De Luca, 1991; Gudas, 1992; Harvat and Jetten, 1999). Clinically, retinoids can reverse premalignant lesions, inhibit the development of second primary tumors, and induce differentiation of acute promyelocytic leukemia (Huang et al., 1988; Sporn et al., 1994; Hong and Sporn, 1997, Lippman and Davies, 1997). These findings led to the use of retinoids in both chemotherapy for and chemoprevention of human cancers; retinoids were approved by the U.S. Food and Drug Administration for the treatment of acute promyelocytic leukemia, Kaposi's sarcoma, and cutaneous T-cell lymphoma.

The effects of retinoids are mediated mainly by two classes of nuclear retinoid receptors: retinoic acid receptors (RARs), which bind to all-trans retinoic acid (RA) and 9-cis RA with similar affinity, and retinoid X receptors (RXRs), which bind to 9-cis RA (Mangelsdorf et al., 1994; Chambon, 1996). These receptors are members of the steroid hormone receptor superfamily, and each one has three subtypes (α, ß, and γ) having distinct amino- and carboxy-terminal domains. The genes RAR-α, RAR-ß, and RAR-γ are localized at chromosomes 17q21, 3p24, and 12q13, respectively, while the genes RXR-α, RXR-ß, and RXR–γ have been mapped to chromosomes 9q34.3, 6p21.3, and 1q22-23, respectively (Mangelsdorf et al., 1994; Chambon, 1996). In addition, the three RARs exhibit a greater nucleotide sequence homology within a subtype across species than between subtypes within a species. This has led to the suggestion that the receptor subtypes have different functions, which have been evolutionarily conserved. In previous studies, *in situ* hybridization revealed that each subtype exhibited a different distribution in adult tissues as well as specific patterns of expression in developing mouse embryos (Dolle et al., 1989; Ruberte et al., 1990, 1991; Xu and Lotan, 1999). In humans, RAR-α mRNA is normally expressed in most tissues; however, RAR-ß expression is more limited, as it is prevalent in neural tissues but barely detectable in skin, while RAR-γ is expressed predominantly in the skin (Mangelsdorf et al., 1994; Chambon, 1996). Also, as a whole, RXRs are widely expressed in adult tissues. RXR-ß expression is found in almost all tissues at similar levels, whereas RXR-α expression is abundant in the liver, kidney, spleen, and skin. In contrast, RXR-expression appears to

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be rather restricted, occurring in abundance mostly in muscle and the brain (Mangelsdorf et al., 1994; Chambon, 1996).

Because nuclear retinoid receptors are the proximate mediators of many effects of retinoids on gene expression (De Luca, 1991; Gudas, 1992; Lotan, 1996; Chambon, 1996; Piedrafita and Pfahl, 1999), it is plausible to assume that changes in the expression and function of these receptors may cause aberrations in the response of cells to RA and thereby alter both the regulation of cell growth and differentiation and the expression of the transformed phenotype (De Luca, 1991; Lotan, 1996; Piedrafita and Pfahl, 1999). Specifically, it has been demonstrated that altered expression of nuclear retinoid receptors is associated with the malignant transformation of human cells (de The, 1996). RAR-α expression, for instance, is abnormally produced by chromosomal translocation during malignant transformation in human promyelocytic leukemia (de The et al., 1990). Also, RAR-ß gene is rearranged in human hepatocellular carcinoma as a result of insertion of a hepatitis B virus sequence (de The et al., 1989). Several studies have also shown that decreased or lost expression of RAR-γ in skin cancers or premalignant lesions (Elder et al., 1991; Finzi et al., 1992). However, most common is the loss of RAR-ß expression, which has been seen in a number of cancers (Xu and Lotan, 1999).

In situ hybridization as a tool for detecting a gene expression pattern in tissue sections

In situ hybridization is a very powerful method of detecting gene expression in tissue specimens, especially when there is no antibody available or when detecting expression of newly cloned genes (Xu and Lotan, 1998). *In situ* hybridization can identify gene expression pattern in tissues; in other words, it can be used to localize the cellular origin of mRNA in tissue sections. This is often the first step in understanding gene function in the study of developmental biology (Albrecht et al., 1998).

The principle underlying *in situ* hybridization is to anneal a labeled DNA or RNA probe to cellular mRNA in tissue sections or cells. *In situ* hybridization is very sensitive, in particular when antisense RNA probes are used. The procedure consists of four major steps: (A) tissue-section preparation, including tissue fixation, dehydration, embedding, and sectioning; (B) probe labeling; (C) annealing of the probe to cellular RNA (hybridization); and (D) visualization of the expression pattern (autoradiography or color reaction).

The probes used in *in situ* hybridization experiments are labeled either with ^{35}[S] NTP or a hapten (digoxigenin or biotin). In the first case, hybridization is detected using autoradiography, while in the second case, antibodies are used to visualize the hapten. Although radioactive probes have been used successfully, they have some disadvantages in the routine analysis of numerous specimens over a long period of time. For example, the generation of a sufficiently intense signal by autoradiography has been shown to take 14 days or longer, the resolution of the signal in individual cells is limited, and the probe has to be prepared often because of the decay of the radioactivity. These disadvantages can be overcome by the development of a nonradioactive labeling method, as using nonradioactive probes provides results in about 2 days (Xu et al., 1994a).

Essential to all *in situ* hybridization analyses is the use of controls that demonstrate probe specificity. A commonly accepted control is the use of sense riboprobes. Hybridization of antisense and sense riboprobes in parallel experiments and under identical conditions is particularly important if a gene appears to be broadly expressed. Once hybridization and washing conditions are established, hybridization using the sense control may be omitted. However, when a new tissue or significantly different embryonic stage is examined, the use of sense controls is strongly recommended (Albrecht et al., 1998).

Having established that the nonradioactive labeling technique provides a rapid but sensitive method of detecting RAR mRNA in formalin-fixed, paraffin-embedded tissue sections, we proceeded to analyze numerous specimens in a variety of tissues, which are described below (see Fig. 1).

Detection of altered expression of nuclear retinoid receptors in head and neck squamous cell carcinoma (HNSCC) and its premalignant lesions

To localize the expression of nuclear retinoid receptors in tissue sections, we used digoxigenin-labeled antisense riboprobes of RAR-α, -ß, and -γ and RXR-α, -ß, and -γ for *in situ* hybridization in histological sections of specimens obtained from both normal volunteers and HNSCC patients (Xu et al., 1994b). All specimens obtained from normal volunteers expressed these receptors. Also, similar levels of RAR-γ, RXR-α, and RXR-ß mRNA were detected in most of the adjacent normal, hyperplastic, dysplastic, and malignant tissues. Specifically, RAR-α mRNA was detected in 94% of adjacent normal and hyperplastic tissues, 87% of dysplastic tissues, and 77% of HNSCCs. In contrast, RAR-ß mRNA was detected in about 70% of adjacent normal and hyperplastic tissues; its expression decreased further to 56% in dysplastic tissues and 35% in HNSCCs. The difference in RAR-ß mRNA level in HNSCCs and adjacent normal tissues was significant (Xu et al., 1994b). These results, together with cell-line data from other studies (Hu et al., 1991; McGregor et al., 1997), indicated that decreased expression of RAR-ß occurs in dysplastic tissues and may be associated with HNSCC development.

Because RAR-ß expression was decreased in dysplastic lesion of the head and neck, we then analyzed tissue specimens of oral premalignant lesions obtained from patients who did not have cancer before and after a

3-month treatment using isotretinoin (Lotan et al., 1995). All the normal tissue specimens expressed RAR-ß mRNA. In contrast, RAR-ß mRNA was detected in only 21 of 52 oral premalignant lesions. The suppression of RAR-ß mRNA expression in the lesions was significant ($p<0.003$). However, 35 of the 39 specimens available for evaluation after treatment expressed RAR-ß mRNA ($p<0.001$). Additionally, all normal and premalignant specimens expressed similar levels of RAR-α, RAR-γ, RXR-α, RXR-ß, and RXR-γ mRNA. The level of RAR-ß mRNA expression increased in the specimens obtained from 18 of 22 patients (82%) who had a response to isotretinoin and from 8 of the 17 (47%) patients who did not have a response ($p=0.04$). These results demonstrated that a selective loss of RAR-ß mRNA expression occurs during the early stages of oral carcinogenesis but that this expression can be restored by isotretinoin treatment in humans. Furthermore, the

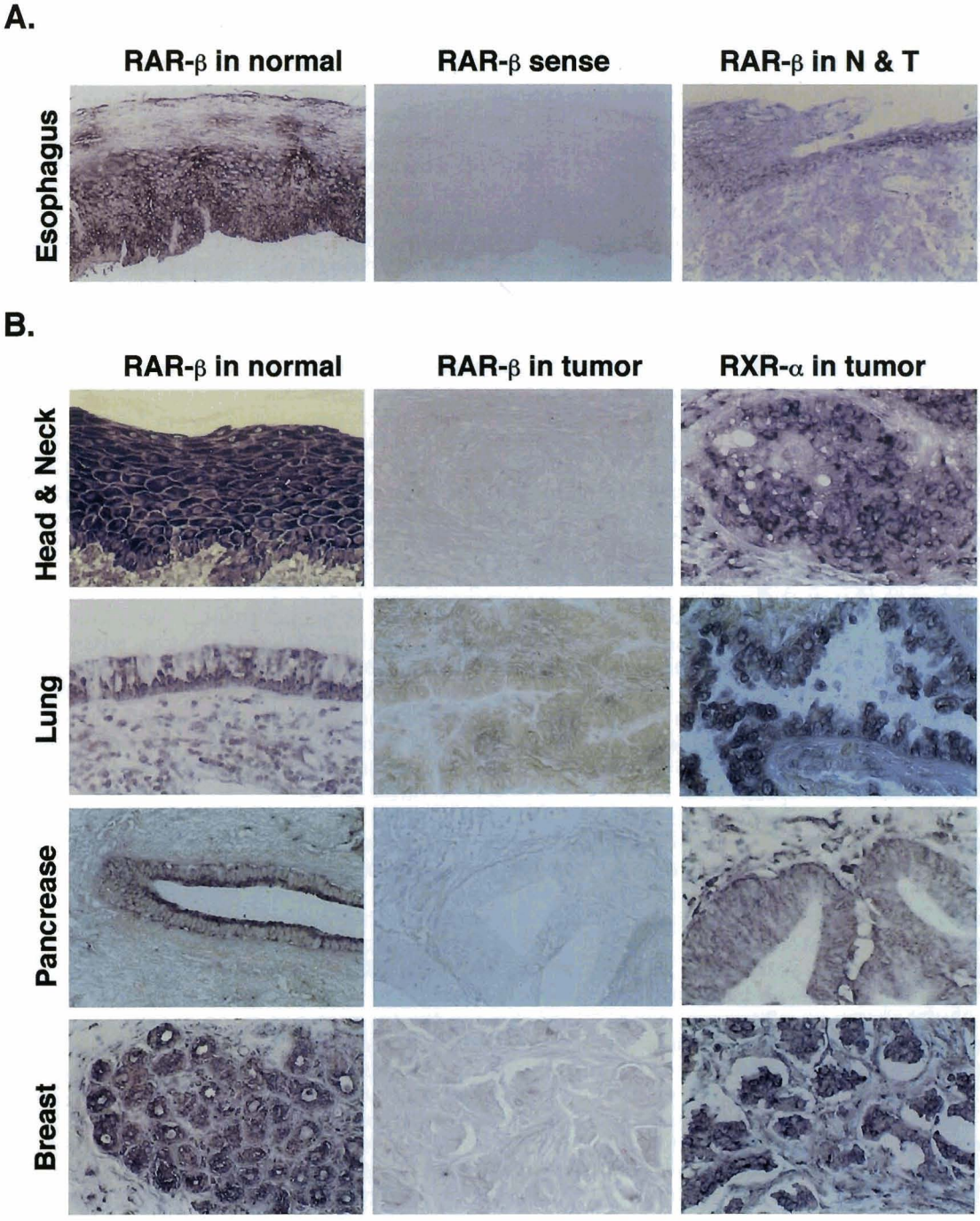

Fig. 1. *In situ* hybridization of formalin-fixed, paraffin-embedded tissue sections obtained from normal and malignant tissue specimens using antisense or sense digoxigenin-labeled riboprobes. **A.** Localization of RAR-ß mRNA in distant and adjacent normal (N) and esophageal cancer (T) sections. **B.** Differential expression of RAR-ß mRNA in normal and malignant tissues from different cancers with RAR-ß antisense probe. RXR-α mRNA was used as a control for intact RNA in the tissue sections.

association between the increase in RAR-ß expression and clinical response suggests that RAR-ß has a role in mediating retinoid response and may be a useful intermediate biomarker in trials of these agents in prevention of oral carcinogenesis (Lotan et al., 1995).

Detection of altered expression of nuclear retinoid receptors in non-small cell lung cancer (NSCLC)

Retinoids are required for the normal growth and differentiation of human bronchial epithelium (Jetten et al., 1992). They are also able to reverse growth of premalignant lesions (Hong and Sporn, 1997) and prevent second primary tumors in some NSCLC patients (Pastorino et al., 1993; Pastorino, 1995). Previous studies using northern blot analysis established that many lung cancer cell lines and primary tumors display abnormal RAR-ß expression (Gebert et al., 1991; Houle et al., 1991, 1993). We extended these studies by using digoxigenin-labeled riboprobes for RAR-α, RAR-ß, RAR-γ, RXR-α, RXR-ß, and RXR-γ for *in situ* hybridization in histological specimens obtained from 79 patients who had undergone pneumonectomy for NSCLC and, as a control, 17 patients having non-lung cancer (Xu et al., 1997a). All 79 samples from the NSCLC patients contained carcinomas; 36 also contained distant normal lung tissues, while 40 contained adjacent normal tissues. All receptors were expressed at very high levels in both control and distant normal specimens. However, in NSCLC, RAR-α, RXR-α, and RXR–γ were expressed at very high levels, whereas RAR-γ expression decreased to 74% in squamous cell carcinoma (SCC) and 70% in adenocarcinoma (ADC), and RXR-ß expression decreased to 73% in SCC and 78% in ADC. In contrast, RAR-ß expression was detected in only 40% of NSCLC ($p<0.0005$). Together with previous cell-line studies (Gebert et al., 1991; Houle et al., 1991, 1993; Nervi et al., 1991; Geradts et al., 1993; Berard et al., 1996), these data suggest that the loss of RAR-ß expression may contribute to lung carcinogenesis, which was further confirmed by Picard et al. (1999).

Next, we determined whether the expression of these receptors is modulated by chemopreventive intervention and analyzed the expression of RAR-ß mRNA in bronchial biopsy samples obtained from heavy smokers at baseline and after 6 months of treatment using isotretinoin or placebo (Xu et al., 1999a). The results showed that RAR-ß mRNA expression was lost in at least one of six biopsy specimens at baseline, in 30 of the 35 subjects (85.7%) in the isotretinoin group and in 24 of the 33 subjects (72.7%) in the placebo group. After a 6-month treatment, the number of RAR-ß-positive subjects in the isotretinoin group increased from 5 to 13 (2.6-fold increase), meaning the percentage of cases having an aberrant RAR-ß expression decreased to 62.9% (22 of 35), which is a statistically significant decrease from the baseline expression level (two-sided McNemar test, $p=0.01$). In the placebo group, there was no significant difference in RAR-ß expression between that at baseline and that at 6 months. Also, RAR-ß expression was not related to smoking status or reversal of squamous metaplasia. Ayoub et al. (1999) reached a similar conclusion, showing that RAR-ß expression is frequently decreased in the bronchial epithelium of smokers and is upregulated at the end of isotretinoin treatment.

Detection of altered expression of nuclear retinoid receptors in breast cancer

Detection of altered expression of nuclear retinoid receptors in breast cancer was analyzed by both Widschwendter et al. (1997) and Xu et al. (1997b). In the first study, Widschwendter and colleagues demonstrated that RAR-α was expressed in all specimens, whereas RAR-γ was expressed in all normal breast tissues but in only 11 of 14 tumors. In addition, RAR-ß expression was found in all distant normal breast tissue specimens, but it was completely lost in 13 of 14 tumors and morphologically adjacent normal tissue specimens. In the second study by Xu et al., the adjacent normal tissue specimens expressed all the nuclear retinoid receptors (RAR-α, RAR-ß, RAR-γ, and RXR-α) in nearly all cases. Expression of RAR-α, RAR-γ, and RXR-α in ductal carcinoma *in situ* (DCIS) was similar to that in normal tissue; however, the expression of RAR-ß was significantly lower in DCIS, especially in poorly differentiated DCIS. Also expression of RAR-γ and RXR-α in invasive carcinoma was similar to that in DCIS and adjacent normal tissue specimens, whereas the expression of RAR-α and RAR-ß decreased to 83.6% and 51.6%, respectively in invasive carcinoma. The decreased expression of RAR-α in invasive carcinoma compared with that in normal tissue as well as in DCIS was somewhat statistically insignificant, whereas the decrease in RAR-ß expression compared with that in normal tissue as well as DCIS in was significant. Together with those of previous studies (Moon et al., 1989; Swisshelm et al., 1994; Seewaldt et al., 1995, 1997), these results clearly indicate a crucial role of RAR-ß in the carcinogenesis of breast cancer.

Detection of altered expression of nuclear retinoid receptors in esophageal cancer

To study retinoid receptor expression in surgical specimens of esophageal cancer, we analyzed expression of RAR-ß and RXR-α mRNA in tissue specimens obtained from 157 esophageal cancer patients and of RAR-α and RAR-γ mRNA in 40 of 157 specimens (Qiu et al., 1999). Of distant normal squamous mucosa (N = 71) and distant columnar mucosa (N = 29) specimens that were also obtained from the 157 patients, 96% of the former expressed RAR-ß. In contrast, RAR-ß was expressed in only 84 of the 157 tumor specimens (54%). The difference in RAR-ß expression between normal and tumor tissue specimens was statistically significant as

determined using the chi-squared test ($p<0.0001$). In addition, RAR-α, RAR-γ, and RXR-α mRNA was expressed at very high percentages (92-98%) in both normal and tumor tissue specimens. Finally, RAR-ß mRNA was expressed in 62% (26 of 42) of well-differentiated SCCs and 54% (27 of 50) of moderately differentiated SCCs, but only 29% (4 of 14) of poorly differentiated SCCs of the esophagus. These results indicate that decreased expression of RAR-ß mRNA may be associated with the development of esophageal cancer.

Our recent *in vitro* data demonstrated that the sensitivity of esophageal cancer cells to RA is associated with the expression and upregulation of RAR-ß. Specifically, cell lines that do not express RAR-ß are resistant to RA (Xu et al., 1999b). Two recent clinical trials of 13-cis RA in patients having advanced esophageal cancer were unsuccessful, showing no response or progression of the disease (Slabber et al., 1996; Enzinger et al., 1999). We propose that these failures may be due, at least in part, to loss of RAR-ß expression. Furthermore, stable transfection of an RAR-ß expression vector into an esophageal cancer cell line, which lacks RAR-ß expression and inducibility by RA and is resistant to RA treatment, decreases cell growth and prolongs doubling time (our unpublished data).

Detection of altered expression of nuclear retinoid receptors in pancreatic cancer

Although RA was previously reported to inhibit the growth of a human pancreatic carcinoma cell line in culture (Rosewicz et al., 1995), we first used digoxigenin-labeled antisense riboprobes of RAR-α, RAR-ß, RAR-γ, and RXR-α for *in situ* hybridization in histological sections of 24 pancreatic carcinoma and 20 adjacent normal tissue specimens (Xu et al., 1996a). All four receptors were detected in adjacent normal pancreatic tissue specimens; RAR-α, RAR-γ, and RXR-α were also detected in all pancreatic carcinoma specimens. In contrast, RAR-ß mRNA was detected in only 67% of the carcinoma specimens and when it is expressed, the level was lower than that in the corresponding adjacent normal tissues. RAR-ß suppression was especially noted in moderately and poorly differentiated cancers. The selective suppression of RAR-ß expression in certain pancreatic carcinomas in vivo may be associated with the development or progression of pancreatic cancer.

Detection of altered expression of nuclear retinoid receptors in cervical cancer

Expression of nuclear retinoid receptors in normal cervical tissues of experimental animals was first reported by Darwiche et al. (1994). In the mouse cervix, both ectocervical and endocervical epithelia have been reported to express RARs and RXRs at varying levels. For example, RAR-α, RAR-γ, RXR-α, and RXR–ß were associated with the cervical stratified squamous subjunctional epithelium. Also the columnar epithelium expressed high levels of RAR-α, RAR-ß, RXR-α, and RXR–ß mRNA, whereas RXR–γ was undetectable in cervical epithelium using *in situ* hybridization (Darwiche et al., 1994). Additionally, RAR-ß, RXR-α, and RXR–ß mRNA was downmodulated in vitamin-A deficient mice. In surgical specimens obtained from cervical cancer patients, RARs were expressed in all adjacent normal cervical epithelia, but their expression was reduced in the lesions, which included cervical SCC and intraepithelial neoplasia I, II, and III (Xu et al., 1999c; our unpublished data).

Detection of altered expression of nuclear retinoid receptors in skin cancer

Several studies have shown a decreased or lost expression of RAR-γ in some skin cancers and premalignant lesions (Elder et al., 1991; Finzi et al., 1992). In addition, *in situ* hybridization experiments using normal sun-exposed epidermis and adjacent SCC specimens have shown positive hybridization with antisense riboprobes for RAR-α and RAR-γ but not RAR-ß in normal epidermis (Xu et al., 1996b). Expression of RAR-α and RAR-γ was not consistent throughout the epidermal layers, however, RAR-α and RAR-γ receptor expression was much higher in the spinous and granular layers than in the basal layer. This pattern of expression was present in all normal skin samples examined. Also, specimens hybridized with antisense riboprobes for RXR-α, RXR-ß, and RXR-γ showed expression of all three receptors in normal epidermis. As with RAR-α and RAR-γ, RXR-α, RXR-ß, and RXR-γ were expressed at higher levels in the spinous and granular layers of the epidermis than in the basal layer.

Additionally, tissue sections from 17 skin SCCs were analyzed for the expression of nuclear retinoid receptors. In comparison with that in adjacent normal epidermis, SCC sections had a noticeable decrease in the expression of RAR-α, RAR-γ, RXR-α, RXR-ß and RXR-γ. Again, RAR-ß expression was not detected in any SCC cells. These results showed that RAR-α and RAR-γ expression was decreased in 16 SCC cases (94%) and RXR-α, RXR-ß, and RXR-γ expression was decreased in 15 (88%), 12 (70%), and 14 (82%) SCC cases, respectively. Increased expression of these receptors was not found in any SCC sections examined.

A low level of nuclear retinoid receptor expression was also seen in basal cell carcinoma (BCC) cases. All BCCs examined were found to express nuclear retinoid receptors at much lower levels than adjacent normal epidermis did (RAR-ß expression was not detected in any BCCs). However, because BCC cells are histologically similar to the nonkeratinizing basal cells of the epidermis, *in situ* staining of the tumor cells was compared with that of the epidermal basal layer. The majority of the BCCs expressed RAR-α, RAR-γ, RXR-

α, RXR-ß, and RXR-γ at levels comparable with that in the basal cells of the epidermis.

Detection of altered expression of nuclear retinoid receptors in prostate cancer

In a recent study of nuclear retinoid receptor expression in the prostate, we first analyzed whether RARs and RXRs are differentially expressed in 8 normal human prostate tissue specimens and 10 prostate cancer tissue specimens and then determined whether oral fenretinide therapy impacts the expression of these receptors in 22 prostate cancers (Lotan et al., 2000). The data showed that RAR-α, RAR-γ, RXR-α, and RXR-γ mRNA was detected in most normal and malignant prostates. However, RAR-ß mRNA expression was found in only 4 of 10 malignant prostates but was present in 7 of 8 normal prostates, which was statistically significant ($p<0.05$). Additionally, RXR–ß mRNA was expressed in 4 of 8 normal prostates but was not expressed in any of the malignant prostates ($p<0.023$). There were no differences in receptor expression between the fenretinide-treated group and a placebo group. Also, RAR-ß and RXR-ß mRNA expression was selectively lost in both prostate cancer and adjacent morphologically normal prostate tissue, supporting the concept of the field of carcinogenesis. One month of oral fenretinide administration (200 mg/day) did not influence the expression of retinoid receptors in prostate cancer.

The use of RAR-ß and RXR-ß expression as a clinical marker for prostate cancer is premature and needs further investigation, but the finding of suppressed expression of RAR-ß and RXR-ß mRNA in histologically normal tissues adjacent to established cancer supports the concept of the field of cancerization, meaning that independent premalignant foci develop concurrently at various rates to form independent primary cancers in the same tissue. If the loss of RAR-ß and RXR-ß expression in normal prostates is proven to be a premalignant feature, its use as a surrogate clinical marker in prostate cancer chemoprevention studies may assume great importance. Because 50% of normal or RXR-ß expression, it may be a more significant correlate.

Use of RARs as biomarkers in clinical prevention trials

RAR-ß expression is suppressed selectively during the early stages of carcinogenesis in the oral cavity. Treatment of leukoplakia using 13-cis RA has been shown to cause a marked increase in the proportion of oral tissue specimens expressing RAR-ß, which was associated with clinical response (Lotan et al., 1995). The same is partially true in bronchial epithelium specimens obtained from tobacco smokers (Ayoub et al., 1999; Xu et al., 1999a). These findings have promoted the use of RAR-ß as an intermediate biomarker because the level of RAR-ß expression decreases during the carcinogenic process and because it is upregulated by a chemopreventive agent (retinoid). Various clinical prevention trials of cancers of the head and neck, lung, skin, prostate, bladder, and breast are under way to further validate RARs as surrogate endpoint biomarkers.

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References

Albrecht U., Lu H.C., Revelli J.P., Xu X-C., Lotan R. and Eichele G. (1998). Studying gene expression on tissue sections using *in situ* hybridization. In: Human genome methods. Adolph K.W. (ed). CRC Press. Boca Raton and New York. pp 93-120.

Ayoub J., Jean-Francois R., Cormier Y., Meyer D., Ying Y., Major P., Desjardins C. and Bradley W.E. (1999). Placebo-controlled trial of 13-cis-retinoic acid activity on retinoic acid receptor-beta expression in a population at high risk: implications for chemoprevention of lung cancer. J. Clin. Oncol. 17, 3546-3552.

Berard J., Laboune F., Mukuna M., Masse S., Kothary R. and Bradley W.E. (1996). Lung tumors in mice expressing an antisense RARbeta2 transgene. FASEB J. 10, 1091-1097.

Chambon P. (1996). A decade of molecular biology of retinoic acid receptors. FASEB J. 10, 940-954.

Darwiche N., Celli G. and De Luca L.M. (1994). Specificity of retinoid receptor gene expression in mouse cervical epithelia. Endocrinology 134, 2018-2025.

De Luca L.M. (1991). Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. FASEB J. 5, 2924-2933.

de The H. (1996). Altered retinoic acid receptors. FASEB J. 10, 955-960.

de The H., Tiollais P. and Dejean A. (1989). Hepatitis B virus and hepatocarcinoma: integration of HBV DNA in the gene coding for the receptor of retinoic acid (editorial). Pathol. Biol. (Paris) 37, 5-6.

de The H., Chomienne C., Lanotte M., Degos L. and Dejean A. (1990). The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. Nature 347, 558-561.

Dolle P., Ruberte E., Kastner P., Petkovich M., Stoner C.M., Gudas L.J. and Chambon P. (1989). Differential expression of genes encoding alpha, beta and gamma retinoic acid receptors and CRABP in the developing limbs of the mouse. Nature 342, 702-705.

Elder J.T., Fisher G.J., Zhang Q.Y., Eisen D., Krust A., Kastner P., Chambon P. and Voorhees J.J. (1991). Retinoic acid receptor gene expression in human skin. J. Invest. Dermatol. 96, 425-433.

Enzinger P.C., Ilson D.H., Saltz L.B., Martin L.K. and Kelsen D.P. (1999). Phase II clinical trial of 13-cis-retinoic acid and interferon-alpha-2a in patients with advanced esophageal carcinoma. Cancer 85, 1213-1217

Finzi E., Blake M.J., Celano P., Skouge J. and Diwan R. (1992). Cellular localization of retinoic acid receptor-gamma expression in normal and neoplastic skin. Am. J. Pathol. 140, 1463-1471.

Gebert J.F., Moghal N., Frangioni J.V., Sugarbaker D.J. and Neel B.G. (1991). High frequency of retinoic acid receptor beta abnormalities in human lung cancer [published erratum appears in Oncogene 1992 Apr;7(4):821]. Oncogene 6, 1859-1868.

Geradts J., Chen J.Y., Russell E.K., Yankaskas J.R., Nieves L. and Minna J.D. (1993). Human lung cancer cell lines exhibit resistance to retinoic acid treatment. Cell Growth Differ. 4, 799-809.

Gudas L.J. (1992). Retinoids, retinoid-responsive genes, cell differentiation, and cancer. Cell Growth Differ. 3, 655-662.

Harvat B. and Jetten A.M. (1999). Growth control by retinoids: regulation of cell cycle progression and apoptosis. In: Retinoids: The biochemical and molecular basis of vitamin A and retinoid action. Handbook of experimental pharmacology. Vol. 139. Nau H. and Blaner W.S. (eds). Springer-Verlag. Berlin. pp 239-276.

Hong W.K. and Sporn M.B. (1997). Recent advances in chemoprevention of cancer. Science 278, 1073-1077.

Houle B., Leduc F. and Bradley W.E. (1991). Implication of RARB in epidermoid (Squamous) lung cancer. Genes Chromosomes Cancer 3, 358-366.

Houle B., Rochette-Egly C. and Bradley W.E. (1993). Tumor-suppressive effect of the retinoic acid receptor beta in human epidermoid lung cancer cells. Proc. Natl. Acad. Sci. USA 90, 985-989.

Hu L., Crowe D.L., Rheinwald J.G., Chambon P. and Gudas L.J. (1991). Abnormal expression of retinoic acid receptors and keratin 19 by human oral and epidermal squamous cell carcinoma cell lines. Cancer Res. 51, 3972-3981.

Huang M.E., Ye Y.I., Chen S.R., Chai J.R., Lu J.X., Zhoa L., Gu L.J. and Wang Z.Y. (1988). Use of all-trans-retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72, 567-572.

Jetten A.M., Nervi C. and Vollberg T.M. (1992). Control of squamous differentiation in tracheobronchial and epidermal epithelial cells: role of retinoids. J. Natl. Cancer Inst. Monogr. 13, 93-100.

Lippman S.M. and Davies P.J. (1997). Retinoids, neoplasia and differentiation therapy. Cancer Chemother. Biol. Response Modif. 17, 349-362.

Lotan R. (1980). Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim. Biophys. Acta 605, 33-91.

Lotan R. (1996). Retinoids and their receptors in modulation of differentiation, development, and prevention of head and neck cancers. Anticancer Res. 16, 2415-2419.

Lotan R., Xu X.C., Lippman S.M., Ro J.Y., Lee J.S., Lee J.J. and Hong W.K. (1995). Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin. N. Engl. J. Med. 332, 1405-1410.

Lotan Y., Xu X.C., Shalev M., Lotan R., Williams R., Wheeler T.M., Thompson T.C. and Kadmon D. (2000). Differential expression of nuclear retinoid receptors in normal and malignant prostates. J. Clin. Oncol. 18, 116-121.

Mangelsdorf D.J., Umesono K. and Evans R.M. (1994). The retinoid receptors. In: The retinoids. Sporn M.B., Roberts A.B. and Goodman D.S. (eds). Raven Press. New York. pp 319-349.

McGregor F., Wagner E., Felix D., Soutar D., Parkinson K. and Harrison P.R. (1997). Inappropriate retinoic acid receptor-beta expression in oral dysplasias: correlation with acquisition of the immortal phenotype. Cancer Res. 57, 3886-3889.

Moon R.C., Pritchard J.F., Mehta R.G., Nomides C.T., Thomas C.F. and Dinger N.M. (1989). Suppression of rat mammary cancer development by N-(4-hydroxyphenyl)retinamide (4-HPR) following surgical removal of first palpable tumor. Carcinogenesis 10, 1645-1649.

Nervi C., Vollberg T.M., George M.D., Zelent A., Chambon P. and Jetten A.M. (1991). Expression of nuclear retinoic acid receptors in normal tracheobronchial cells and in lung carcinoma cells. Exp. Cell Res. 195, 163-170.

Pastorino U. (1995). Lung cancer chemoprevention. Cancer Treat. Res. 72, 43-74.

Pastorino U., Infante M., Maioli M., Chiesa G., Buyse M., Firket P., Rosmentz N., Clerici M., Soresi E. and Valente M. (1993). Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J. Clin. Oncol. 11, 1216-1222.

Picard E., Seguin C., Monhoven N., Rochette-Egly C., Siat J., Borrelly J., Martinet Y., Martinet N. and Vignaud J.M. (1999). Expression of retinoid receptor genes and proteins in non-small-cell lung cancer. J. Natl. Cancer Inst. 91, 1059-1066.

Piedrafita F.J. and Pfahl M. (1999). Nuclear retinoid receptors and mechanisms of action. In: Retinoids: The Biochemical and molecular basis of vitamin A and retinoid action. Handbook of experimental pharmacology. Vol. 139. Nau H. and Blaner W.S. (eds). Springer-Verlag. Berlin. pp 153-184.

Qiu H., Zhang W., El-Naggar A.K., Lippman S.M., Lin P., Lotan R. and Xu X.C. (1999). Loss of retinoic acid receptor-beta expression is an early event during esophageal carcinogenesis. Am. J. Pathol. 155, 1519-1523.

Rosewicz S., Stier U., Brembeck F., Kaiser A., Papadimitriou C.A., Berdel W.E., Wiedenmann B. and Riecken E.O. (1995). Retinoids: effects on growth, differentiation, and nuclear receptor expression in human pancreatic carcinoma cell lines. Gastroenterology 109, 1646-1660.

Ruberte E., Dolle P., Krust A., Zelent A., Morriss-Kay G. and Chambon P. (1990). Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis. Development 108, 213-222.

Ruberte E., Dolle P., Chambon P. and Morriss-Kay G. (1991). Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. Development 111, 45-60.

Seewaldt V.L., Johnson B.S., Parker M.B., Collins S.J. and Swisshelm K. (1995). Expression of retinoic acid receptor beta mediates retinoic acid-induced growth arrest and apoptosis in breast cancer cells. Cell Growth Differ. 6, 1077-1088.

Seewaldt V.L., Kim J.H., Caldwell L.E., Johnson B.S., Swisshelm K. and Collins S.J. (1997). All-trans-retinoic acid mediates G1 arrest but not apoptosis of normal human mammary epithelial cells. Cell Growth Differ. 8, 631-641.

Slabber C.F., Falkson G., Burger W. and Schoeman L. (1996). 13-cis-retinoic acid and interferon alpha-2a in patients with advanced esophageal cancer: a phase II trial. Invest. New Drugs 14, 391-394.

Sporn M.B., Roberts A.B. and Goodman D.S. (1994). The Retinoids: Biology, chemistry, and medicine. 2nd ed. Raven Press. New York.

Swisshelm K., Ryan K., Lee X., Tsou H.C., Peacocke M. and Sager R. (1994). Down-regulation of retinoic acid receptor beta in mammary carcinoma cell lines and its up-regulation in senescing normal mammary epithelial cells. Cell Growth Differ. 5, 133-141.

Widschwendter M., Berger J., Daxenbichler G., Muller-Holzner E.,

Widschwendter A., Mayr A., Marth C. and Zeimet A.G. (1997). Loss of retinoic acid receptor beta expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. Cancer Res. 57, 4158-4161.

Xu X.C. and Lotan R. (1998). Nonisotopic *in situ* hybridization for the detection of nuclear retinoid receptor transcripts in tissue sections. Methods Mol. Biol. 89, 233-246.

Xu X.C. and Lotan R. (1999). Aberrant expression and function of retinoid receptors in cancer. In: Retinoids: the biochemical and molecular basis of vitamin A and retinoid action. Handbook of experimental pharmacology. Vol. 139. Nau H. and Blaner W.S. (eds). Springer-Verlag. Berlin. pp 323-343.

Xu X.C., Clifford J.L., Hong W.K. and Lotan R. (1994a). Detection of nuclear retinoic acid receptor mRNA in histological tissue sections using nonradioactive *in situ* hybridization histochemistry. Diagn. Mol. Pathol. 3, 122-131.

Xu X.C., Ro J.Y., Lee J.S., Shin D.M., Hong W.K. and Lotan R. (1994b). Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues. Cancer Res. 54, 3580-3587.

Xu X.C., Stier U., Rosewicz S., El-Naggar A.K. and Lotan R. (1996a). Selective suppression of nuclear retinoic acid receptor beta gene expression in human pancreatic cancers. Int. J. Oncol. 8, 445-451.

Xu X.C., Wong W.Y.L., Goldberg L.H., Baer S., Wolf J.E. and Lotan R. (1996b). Suppression of nuclear retinoid receptor expression in squamous cell carcinomas of the skin. Proc. Amer. Assoc. Cancer Res. 37, 1588.

Xu X.C., Sozzi G., Lee J.S., Lee J.J., Pastorino U., Pilotti S., Kurie J.M., Hong W.K. and Lotan R. (1997a). Suppression of retinoic acid receptor beta in non-small-cell lung cancer in vivo: implications for lung cancer development . J. Natl. Cancer Inst. 89, 624-629.

Xu X.C., Sneige N., Liu X., Nandagiri R., Lee J.J., Lukmanji F., Hortobagyi G., Lippman S.M., Dhingra K. and Lotan R. (1997b). Progressive decrease in nuclear retinoic acid receptor beta messenger RNA level during breast carcinogenesis. Cancer Res. 57, 4992-4996.

Xu X.C., Lee J.S., Lee J.J., Morice R.C., Liu X., Lippman S.M., Hong W.K. and Lotan R. (1999a). Nuclear retinoid acid receptor beta in bronchial epithelium of smokers before and during chemoprevention. J. Natl. Cancer Inst. 91, 1317-1321.

Xu X.C., Liu X., Tahara E., Lippman S.M. and Lotan R. (1999b). Expression and up-regulation of retinoic acid receptor-beta is associated with retinoid sensitivity and colony formation in esophageal cancer cell lines. Cancer Res. 59, 2477-2483.

Xu X.C., Mitchell M.F., Silva E., Jetten A. and Lotan R. (1999c). Decreased expression of retinoic acid receptors, transforming growth factor beta, involucrin, and cornifin in cervical intraepithelial neoplasia. Clin. Cancer Res. 5, 1503-1508.

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