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Differential expression of the α2 chain of the interleukin-13 receptor in metastatic human prostate cancer ARCaP$_M$ cells

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Abstract

Background—The α2 chain of the interleukin-13 receptor (IL13Rα2) is a high affinity receptor and a candidate target for cytotoxic killing of cancer cells. Availability of a human prostate cancer cell line with high level of IL13Rα2 expression will facilitate the development of therapeutic modalities.

Methods—ARCaP$_E$ and ARCaP$_M$ human prostate cancer cell lines were subjected to comparative analyses of gene expression. Expression of the IL13Rα2 protein was confirmed by western blotting and immunostaining. IL13Rα2 proteins in xenograft tumors and clinical human prostate cancer specimens were detected by specific antibodies. LNCaP prostate cancer cells stably transfected with IL13Rα2 were examined for accelerated growth in athymic mice.

Results—We found that IL13Rα2 proteins could be detected in both the ARCaP$_E$ and ARCaP$_M$ cells, but the expression level in ARCaP$_M$ was more than 17-folds higher than in ARCaP$_E$ cells. Importantly, the ARCaP lineage represented the only human prostate cancer cell line that expresses IL13Rα2 proteins at the level detectable by western blotting. Expression of IL13Rα2 was accompanied by resistance to the anti-tumor activity of interleukin-13 (IL-13). ARCaP cells were found to be insensitive to growth inhibition upon IL-13 treatment, while overexpression of IL13Rα2 in LNCaP cells promoted intratibial tumor growth in athymic mice.

Conclusions—Differential IL13Rα2 expression may account for the high tumorigenic and metastatic potential of ARCaP$_M$ cells. The unique expression of IL13Rα2 makes ARCaP lineage an attractive model for evaluating the targeting efficacy of therapeutic agents based on IL13Rα2 protein expression.

Keywords

Interleukin-13 receptor α2; prostate cancer; differential gene expression; anti-tumor activity; gene targeting

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Introduction

Prostate cancer is the most diagnosed malignancy and the second leading cause for cancer death in men in Western nations (1). Diagnosed initially as androgen-dependent neoplasia, prostate cancer frequently progresses to androgen-independence and metastasis to distant sites, preferentially in the bone. Clinically available therapies are mostly systematic and palliative. Effective therapies for metastatic prostate cancer should have specific effects on cancer cells, and be able to eradicate infiltrating tumor cells without affecting the normal cells.

Targeted therapies block the growth and spread of prostate cancer cells by cytotoxic selectivity to tumor markers. Surface proteins found specifically on cancer cells are ideal for such targeting. In this regard, the α2 chain of interleukin-13 receptor (IL13Rα2) has been shown to be a promising target. IL13Rα2, for instance, could be targeted with IL13-PE38QQR, a recombinant cytotoxin containing a mutated Pseudomonas exotoxin (2-4), while a transfected IL13Rα2 expression rendered prostate cancer cells sensitive to the cytotoxic killing (5). Identification of a human prostate cancer cell line with high level of endogenous IL13Rα2 expression may facilitate the development of targeted therapies.

We have used human $ARCaP_E$ and $ARCaP_M$ prostate cancer cells as models to identify prostate cancer biomarkers (6,7). Under regular culture conditions, $ARCaP_E$ cells display epithelial morphology. In xenograft tumor formation assays in athymic mice, $ARCaP_E$ cells show limited tumorigenic capacity and metastatic potential. However, $ARCaP_E$ cells could undergo epithelial to mesenchymal transition to adopt mesenchymal stromal morphology and behavior upon interaction with the host tumor microenvironment during the xenograft tumor formation (7). The resultant $ARCaP_M$ cells show increased tumorigenic capacity and acquired metastatic potency. $ARCaP_E$ and the derivative $ARCaP_M$ cells thus form a lineage-related model simulating the progression and metastasis of human prostate cancer.

In the present work, we used gene expression profiling to identify a unique expression of the IL13Rα2 in $ARCaP_E$ and $ARCaP_M$ cells. Among a panel of human prostate cancer cell lines, we found that the IL13Rα2 protein was detected only in the $ARCaP_E$ lineage, where differential expression resulted in a much higher IL13Rα2 protein level in the $ARCaP_M$ cells. Molecular studies indicated that IL13Rα2 antagonized the anti-tumor activity of interleukin-13 (IL-13). This study thus provides a unique model for studying IL13Rα2 as a biomarker in the therapeutic targeting of human prostate cancer.

Materials and Methods

Cell culture

The culture of the human prostate cancer cell lines has been reported (8). The HMC-1 human mast cell line was a kind gift from Dr. Butterfield (Mayo Clinic, Rochester, MN), and were cultured in Iscove's Modified Dulbecco's Medium (Invitrogen, Carlsbad, CA) containing 5% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT), penicillin (100 U/ml) and streptomycin (100 μg/ml). All the cells were cultured at 37°C with 5% CO_2 in a humidified incubator.

Comparative gene expression analysis

The protocol used for microarray gene expression analysis was reported previously (8). In a separate study to compare gene expression using membrane-based gene array, two pairs of Human Cytokine Arrays V2.0 (Sigma-Genosys, Woodlans, TX) were used following the manufacturer's recommended protocol. Total RNA (5 μg) was used to purify mRNA with the PolyATtract mRNA isolation system (Promega, Madison, WI). The mRNA was used in

first-strand cDNA synthesis with α-^{32}P-dCTP incorporation to synthesize probes. After hybridization and stringent washing, specific signals were detected with autoradiography.

RT-PCR analysis and cloning

The protocol used for RT-PCR was previously reported (8). Primer pairs used in RT-PCR analyses are listed in Table 1. Primers used in RT-PCR cloning of human IL13Rα2 cDNA were 5'-CTACTCGAGCTATGGCTTTCGTTTGCTTGGCTATC-3' and 5'-TCATCTAGATCATGTATCACAGAAAAATTCTG-3'. PCR products were cloned to pGEM-T Easy vector (Promega, Madison, WI) and confirmed by DNA sequencing analysis. Following digestion with XhoI and XbaI restriction enzymes, the cDNA was transferred to the pcDNA3.1(+) eukaryotic expression vector.

Transfection

The protocol used for transfection and isolation of transfected clones was previously reported (9). To assess transfection efficiency, the pAsRed2 eukaryotic expression vector (Clontech, Mountain View, CA) was used in co-transfection. Efficiency of the co-transfection was determined by red fluorescence microscopy. To isolate stably transfected clones, the cells were subjected to G418 selection (400 μg/ml) for two weeks. Surviving cells were subjected to further cloning with the limited dilution method.

Western blotting

The western blotting protocol has been reported (8). The MAB614 monoclonal antibody to human IL13Rα2 were purchased from R&D Systems (Minneapolis, NM). The YY-23Z monoclonal antibody to IL13Rα2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). In western blottings, primary antibodies were used at 1 μg/ml.

Immunocytochemical and immunohistochemical staining

The protocols used for staining cells on chamber slides and formalin-fixed paraffin-embedded prostate tissue specimens have been reported (7). The source of human prostate cancer specimens was described previously (8,9). In Immunocytochemical and immunohistochemical stainings, the monoclonal antibodies to IL13Rα2 were used at 0. 5 μg/ml.

Cell proliferation assay

The protocol for measuring cell proliferation with MTT conversion has been reported (10). In this study, cells were plated onto 96-well plate at a density of 2.5×10^4/100 μl medium in each well for 24 hours. The cells were then treated in triplicate with interleukin-4 (IL-4) or IL-13 (R&D Systems) for 72 hours, followed by MTT assay.

Xenograft tumor formation

The protocol used for intratibial inoculation has been reported (7). In this study, level of Prostate Specific Antigen (PSA) in tumor-bearing animals was used to assess intratibial tumor growth (11). Animal blood was obtained by retro-orbital bleeding and the concentration of PSA was determined in triplicate with the PSA ELISA kit (United Biotech Inc., Mountain View, CA) following the manufacturer's recommended protocol.

Statistical analysis

Student's *t*-test was used. Level of statistical significance between control and treated groups was set at $P < 0.05$.

Results

1. Differential expression of IL13Rα2 between $ARCaP_E$ and $ARCaP_M$ cells

$ARCaP_M$ cells display mesenchymal stromal cell morphology and increased tumorigenicity, different from the lineaged $ARCaP_E$ cells (7). We conducted two separate expressional comparisons between these cells in order to search for candidate biomarkers for the acquired metastatic potential of the derivative $ARCaP_M$ cells. First-strand cDNA probes synthesized with mRNA of the $ARCaP_E$ and $ARCaP_M$ cells were used. In one study, expression profiles of human genes in the Prostate Expression Database (12) were compared. In a complementary study, Human Cytokine Arrays were used to identify surface receptors that are expressed at a higher level in $ARCaP_M$ than in $ARCaP_E$ cells. This membrane based array contained 847 human cytokines, chemokines, growth factors, and the cognate receptors in the cDNA form. Both of the expressional comparison analyses identified that IL13Rα2 was differentially expressed in the $ARCaP_M$ cells (Figure 1A).

The differential expression was confirmed by western blotting using specific antibodies to human IL13Rα2 protein (Figure 1B). A densitometric quantification of two separate results suggested that $ARCaP_M$ cells expressed 17 fold more IL13Rα2 proteins than did the $ARCaP_E$ cells. IL13Rα2 precursor has a calculated molecular mass of 44.2 kD. Under reducing conditions, it was detected as a cluster of bands between 44 kD to 65 kD. The bands with increased sizes probably resulted from post-translational modification, since this protein contains multiple consensus sites for N-glycosylation and has been shown to be highly glycosylated (13,14).

An interesting finding from this study was the unique expression of IL13Rα2 in the ARCaP lineage. When a panel of commonly used human prostate cancer cell lines were examined for IL13Rα2 proteins, only cells of the ARCaP lineage showed specific signals (Figure 1B). After repeated western blotting analyses with two different antibodies, we concluded that ARCaP was the only human prostate cancer cell lineage that expressed IL13Rα2 proteins at a level detectable by western blotting.

The differential expression of IL13Rα2 could also be seen at the cellular level and in xenograft tumors following immunostaining. No specific signals were detected in cells of the LNCaP and the lineaged C4-2 and C4-2B cells, nor in PC-3, PC-3M and DU145 cells (Figure 2A and data not shown). Specific stains were seen in $ARCaP_E$ cells, where a low but discernible level was distributed in cytoplasm. In comparison, intense stains were seen in $ARCaP_M$ cells (Figure 2A). A similar expression pattern was seen in xenograft tumor specimens. $ARCaP_E$ subcutaneous tumors showed weak but specific signals, whereas $ARCaP_M$ subcutaneous tumors displayed strong cytoplasmic staining (Figure 2B). $ARCaP_M$ cells have a high propensity to metastasize to adrenal gland and bone (7). In these metastatic tumors, IL13Rα2 was detected as intensely stained clusters specifically in the tumor zone (Figure 2B). In parallel studies no specific signals were detected in xenograft tumors of the C4-2 cells (data not shown). These data demonstrate that among the human prostate cancer cell lines examined, IL13Rα2 is expressed exclusively in the ARCaP lineage, where it is differentially expressed in the highly tumorigenic and metastatic $ARCaP_M$ subline.

We determined that IL13Rα2 was abnormally expressed in clinical prostate tumor specimens. The two normal prostate specimens were found to be negative for IL13Rα2 (data not shown). The two primary tumor specimens both were detected with IL13Rα2 in cancerous glands (Figure 2C), while three of the four bone metastatic tumor specimens examined were stained strongly (Figure 2C). These results suggested that IL13Rα2 was frequently expressed in prostate cancers.

2. Differential expression of the IL-13 receptor subunits

The IL-13 cytokine exerts anti-inflammatory and anti-tumor effects by activating the IL-13 receptor complex, which is comprised of heterodimeric subunits of interleukin-13 receptor α1 (IL13Rα1) and interleukin-4 receptor α (IL4Rα) (14). Together with the common γ chain of interleukin-2 receptor (IL2Rγc), these subunits are also involved in the IL-4 signaling (15,16). IL-13 seemed to have opposite effects on different cancer cells, being able to inhibit growth of breast cancer (17) and renal carcinoma cells (18), or to promote proliferation in some other cancers (4,19). As a high affinity receptor, IL13Rα2 modulates signaling of the IL-13 receptor complex, and may antagonize the IL-13 anti-tumor activity (20,21).

In order to evaluate the function of IL13Rα2 in human prostate cancer cell lines, we performed RT-PCR analysis to examine expression of the receptor subunits. The HMC-1 human mast cells were used as a positive control, since these cells are sensitive to IL-13 and may express subunits of the IL-13 receptor (22). A high level of IL13Rα2 transcripts was detected in $ARCaP_M$ (Figure 3A), while a reduced level was found in $ARCaP_E$ cells, agreeing with the western blotting results (Figure 1). None of the human prostate cancer lines expressed detectable levels of IL-13 or IL-4 cytokines. IL13Rα1 could be detected in all the cell lines, albeit at varied levels. In the ARCaP lineage, $ARCaP_E$ appeared to produce more IL13Rα1 than the $ARCaP_M$ cells, opposite to the pattern of IL13Rα2 expression (Figure 3A). IL4Rα was expressed uniformly, while levels of the IL2Rγc were varied among prostate cancer cell lines and were much reduced compared to the level in HMC-1 cell line. These results suggested that prostate cancer cell lines should respond to IL-13 signaling, since these cells could express IL13Rα1 and IL4Rα. On the other hand, the ARCaP lineage may have different responses to IL-13 treatment, because these cells expressed an additional IL13Rα2 subunit.

This analysis additionally revealed an interesting feature of IL13Rα2 expression in the PC-3 lineage. Although little IL13Rα2 proteins were detected in PC-3 and PC-3M cells (Figure 1B), IL13Rα2 transcripts could be amplified from these cells (Figure 3A). We used RT-PCR cloning and DNA sequencing to examine IL13Rα2 coding sequences in PC-3 and $ARCaP_M$ cells. Both the coding sequences were identical to that reported in GenBank (accession number NM_000640). These results suggested a discrepancy between transcription and protein production in PC-3 lineaged cells.

3. Differential response of human prostate cancer cells to IL-13 treatment

We then assessed whether the highly expressed IL13Rα2 in $ARCaP_M$ cells was functional by evaluating differential response to IL-13 treatment. In three separate experiments, treatment with the IL-13 cytokine resulted in growth inhibition in LNCaP and PC-3 cells (Figure 3B). In parallel studies, IL-13 did not inhibit growth of the $ARCaP_M$ cells, suggesting that IL-13 anti-tumor activity was compromised, and IL13Rα2 may be accountable for the neutralizing effect.

As a T helper 2 cytokine, IL-13 shares structural and functional features with IL-4 (15,16). The biological activity of IL-13 can be distinguished from that of the IL-4. When both IL-13 and IL-4 were used to treat prostate cancer cells, we observed a preferential growth inhibition by IL-13 over IL-4 (Figure 3B). Further investigation is needed to elucidate the mechanism underlying the differential anti-tumor activity of these cytokines.

4. IL13Rα2 renders cancer cells insensitive to the IL-13 anti-tumor activity

To confirm the antagonism of IL13Rα2 to IL-13 anti-tumor activity, we cloned the coding sequence for IL13Rα2 to the pcDNA3.1(+) eukaryotic expression vector. The construct was transfected to prostate cancer cell lines for isolating clones stably expressing the protein.

IL13Rα2 protein was detected in LNCaP cells 48 hours after transfection (Figure 4A), and multiple clones stably expressing IL13Rα2 protein were isolated by limited dilution after antibiotic selection. In contrary, when the same construct was transfected to PC-3 cells, little IL13Rα2 protein could be detected after transient transfection (Figure 4A), and no stable clones could be isolated expressing the IL13Rα2 protein following three repeated transfections. To determine whether PC-3 cells were unable to produce a stable level of IL13Rα2 proteins, the IL13Rα2 expression construct was co-transfected together with the pAsRed2 eukaryotic expression vector. The co-transfetion showed a transfection efficiency of about 42% based on the ratio of red fluorescent cells. Nevertheless, none of the red fluorescent PC-3 clones isolated expressed any IL13Rα2 protein (data not shown). These results suggested that PC-3 prostate cancer cells could not produce IL13Rα2 protein up to a level detectable by western blotting.

We carried on the study by using LNCaP clones expressing the transfected IL13Rα2. Three clones were randomly picked and used in the study (Figure 4B). Compared to the vector-transfected control clones, all three IL13Rα2-expressing clones showed slightly accelerated growth rate, indicating that IL13Rα2 expression promotes cell proliferation (Figure 4C). Upon treatment with IL-13, there was a significant reduction in growth rate in the control cells. Conversely, all three IL13Rα2-expressing clones became insensitive to IL-13 induced growth inhibition (Figure 4C). IL13Rα2, therefore, played a role in antagonizing the anti-tumor activity of the IL-13 cytokine.

5. IL13Rα2 facilitates tumor formation

$ARCaP_M$ cells, which expressed a high level of IL13Rα2, are known to have high tumorigenic and metastatic capabilities (7). We investigated whether a high level of IL13Rα2 in LNCaP cells could similarly induce an accelerated tumor formation *in vivo*. Since all three clones showed similar insensitivity to IL-13 treatment (Figure 4B), one representative clone was used in the *in vivo* analysis. Cells from the clone were inoculated to the tibias of six athymic mice.

Tumor cell growth was assessed by biweekly PSA measurement (11). This study demonstrated that the growth rate of the intratibial tumors was significantly enhanced. Compared to the control animals, which were inoculated with LNCaP cells transfected with the pcDNA3.1(+) vector, mice inoculated with IL13Rα2-expressing LNCaP cells were found to have significantly increased PSA in circulation, especially in the later phase 10 weeks after the inoculation (Figure 4D). The study was terminated at the 13th week post-inoculation, due to tumor hemorrhage and tumor rupture in the experimental group.

Discussion

In this study comparing gene expression between the lineaged $ARCaP_E$ and $ARCaP_M$ cells, we found a high level of IL13Rα2 protein expressed in the highly tumorigenic and metastatic $ARCaP_M$ prostate cancer cells (Figures 1 and 2). Elevated IL13Rα2 seemed a frequent phenomenon in prostate cancer, as five of the six clinical prostate cancer specimens were detected with abnormal IL13Rα2 expression (Figure 2C). On the other hand, the results of this study suggest that IL-13 has anti-tumor activity, since treatment with IL-13 inhibited growth of the LNCaP and PC-3 cells (Figures 3B and 4C). In contrary, IL13Rα2 may play dual role in promoting cancer cell growth and tumor formation. IL13Rα2 expression renders cancer cells resistant to the IL-13 anti-tumor activity, indicating that IL13Rα2 antagonizes IL-13 function. LNCaP cells overexpressing IL13Rα2 proteins showed accelerated tumor formation in athymic mice (Figure 4D). This could be in part due to increased resistance to IL-13 of the mouse host, since IL-13 has cross-species activity in human and mouse (23,24). Interestingly, LNCaP clones overexpressing this protein grew

faster *in vitro* than the control clones (Figure 4C), suggesting that IL13Rα2 could also function directly to promote cancer cell growth and tumor formation. In this respect, IL13Rα2 has been recently shown to have signaling capability (25). Further investigation is warranted to elucidate the mechanism of IL13Rα2 in promoting cancer cell growth and tumor formation.

As a high affinity receptor, IL13Rα2 has been shown to be a promising target for the cytotoxic killing of cancer cells (26-31). On the other hand, few studies have been devoted to evaluate the expression of IL13Rα2 at the protein level. Although this protein may be abnormally expressed in prostate cancer specimens (Figure 2C), a human prostate cancer cell line with high IL13Rα2 protein expression has not been identified previously. Our study demonstrates that the ARCaP lineage expresses substantial amounts of functional IL13Rα2 protein (Figures 1, 2, and 3). The multiple banding detected in western blotting suggests an extensive post-translational modification of the IL13Rα2 protein. We have so far used two different antibodies to detect IL13Rα2 proteins and similar banding patterns were obtained. Importantly, when the LNCaP cells were transfected with the IL13Rα2 eukaryotic expression construct, these antibodies detected a banding pattern similar to the endogenous IL13Rα2 detected in $ARCaP_M$ cells (Figures 4A and 4B). Importantly, among the human prostate cancer cell lines examined, ARCaP cells exclusively express IL13Rα2. This study is the first to identify a human prostate cancer cell line with high level IL13Rα2 protein expression.

In PC-3 lineaged cells, there is a discrepancy between the mRNA level and protein amount of IL13Rα2 (Figure 3A). Whereas substantial amounts of mRNA could be detected by RT-PCR, no IL13Rα2 protein has been detected by western blotting (Figure 1). We did not find sequence abnormalities in the coding region of the IL13Rα2 from PC-3 cells. Transfection with the same expression construct was followed by detection of the IL13Rα2 protein in LNCaP but not in PC-3 cells (Figures 4A and 4B). Although seen with substantial amount of IL13Rα2 mRNA, PC-3 cells remained sensitive to IL-13-induced growth inhibition (Figure 3). These observations suggest that either the coding region of the IL13Rα2 harbors certain features prohibiting synthesis of the protein in PC-3 cells, or synthesized IL13Rα2 protein is efficiently degraded in this cell line. Further investigation is needed to define the cause of this discrepancy.

The IL-13 cytokine is a participant in cellular immunity to tumor formation (20,21). IL-13 activates the IL-13 receptor complex to elicit signaling of the JAK1/STAT6 pathway (15,16), which may promote cell differentiation and inhibit tumor growth. The biological function of IL13Rα2 is less clear. Whereas it has been considered a decoy receptor (32), recent studies suggest that IL13Rα2 may have signaling potential (25). As a high affinity receptor, it may be involved in fine-tuning the availability of the IL-13. High level expression of this receptor in tumor cells would result in a situation in which anti-tumor activity of the IL-13 is compromised, leading to the nullification of cellular immunity and the occurrence of tumor escape. In this sense, targeting tumor IL13Rα2 is a highly specific and efficient strategy, and the ARCaP lineaged cells are valuable tools for the development of therapeutic targeting modalities.

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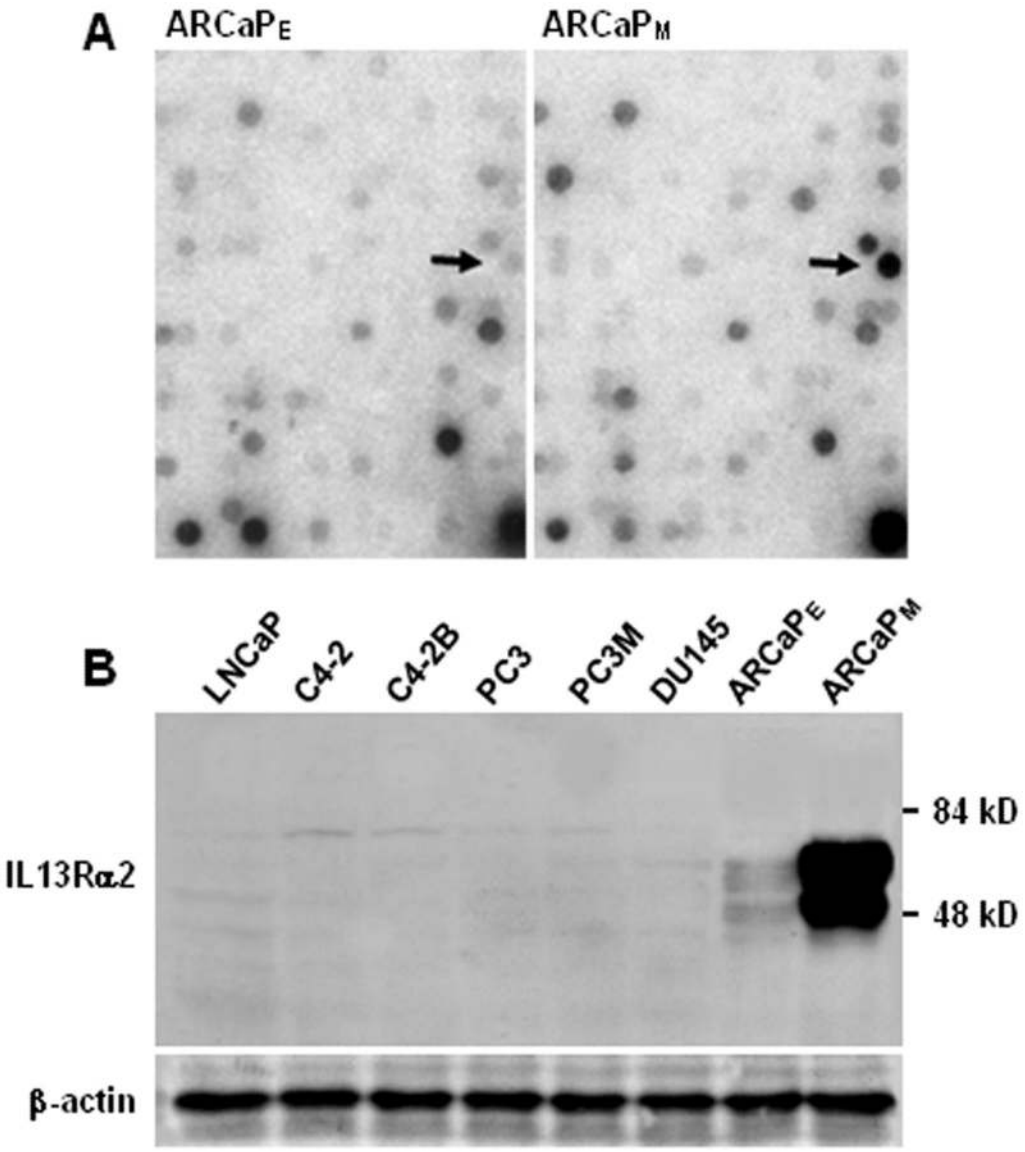

Figure 1. Differential IL13Rα2 expression in the ARCaP lineage

A, $ARCaP_E$ and $ARCaP_M$ cells were subjected to expression comparison with the Human Cytokine Arrays. A selected area of the array data is shown. Compared to $ARCaP_E$ cells, increased IL13Rα2 transcription (arrow) in $ARCaP_M$ cells was identified. **B**, in the upper panel, proteins from each prostate cancer cell line were subjected to western blotting for IL13Rα2 with the MAB614 antibody. In the lower panel, β-actin was used to show equal loading. This result is representative of three separate western blottings.

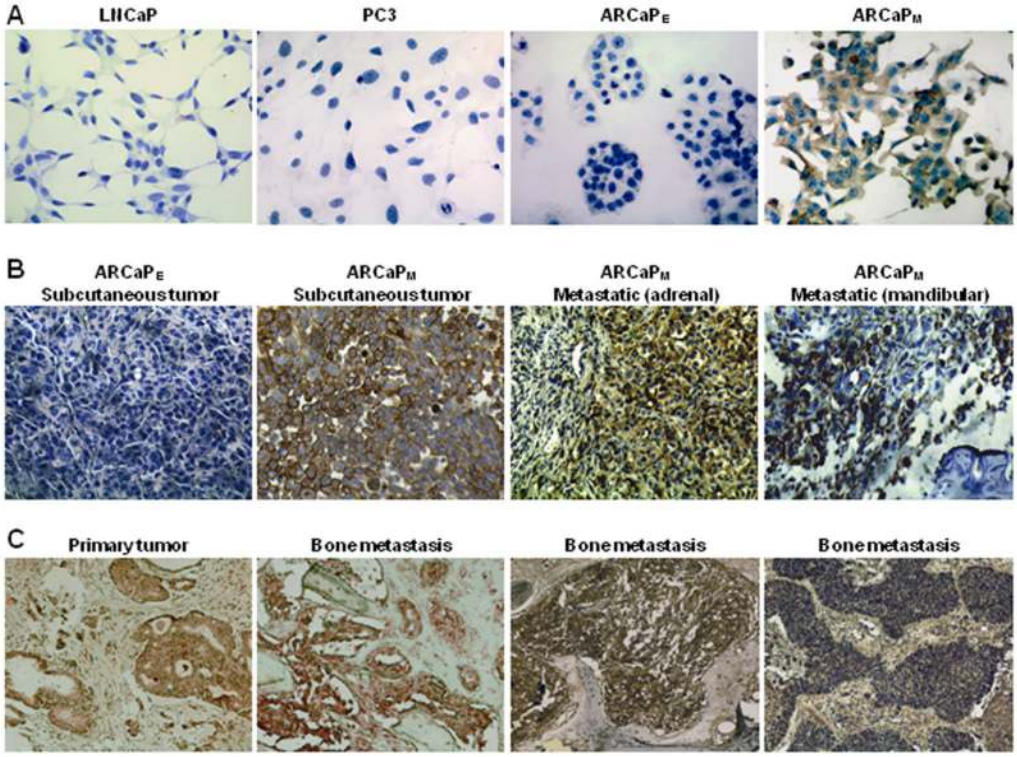

Figure 2. Differential IL13Rα2 expression in prostate cancer cells and prostate cancer specimens

A, prostate cancer cells were subjected to immunocytochemical staining for IL13Rα2. Each result is representative of two separate staining experiments. The images are shown at 200x magnification. **B**, xenograft tumors were stained for IL13Rα2. The level of IL13Rα2 in subcutaneous ARCaP$_E$ tumors was compared to subcutaneous ARCaP$_M$ tumors. Tumors of the adrenal and mandibular metastases were resulted from intracardiac inoculation of ARCaP$_M$ cells (7). All images are shown at 100x magnification. **C**, human prostate specimens were stained for IL13Rα2. Representative results from the staining of primary and bone metastatic tumors are shown. The images are shown at 40x magnification.

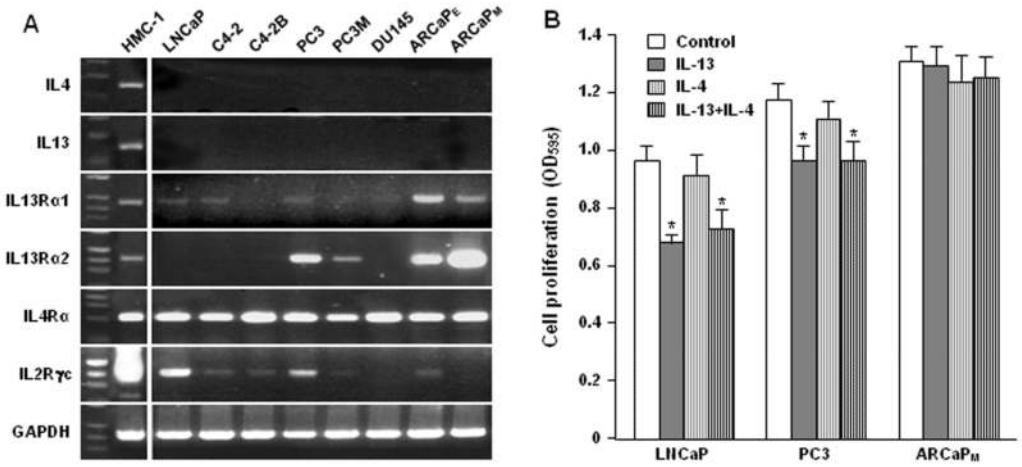

Figure 3. Expression of IL-13 receptor subunits in prostate cancer cells and their differential responses to IL-13 treatment

A, expression of cytokines and receptor subunits was determined by RT-PCR. Primers used in the assay and expected sizes of the PCR products are listed in Table 1. The results are representative of two separate experiments. **B**, LNCaP, PC-3 and ARCaP$_M$ cells were treated with IL-13 (25 ng/ml) or IL-4 (25 ng/ml) for 72 hours in a cell proliferation assay with the MTT conversion method. The results are representative of three separate experiments. For each cell line, an asterisk indicates statistical significance ($P < 0.05$) compared to control group of the cell line.

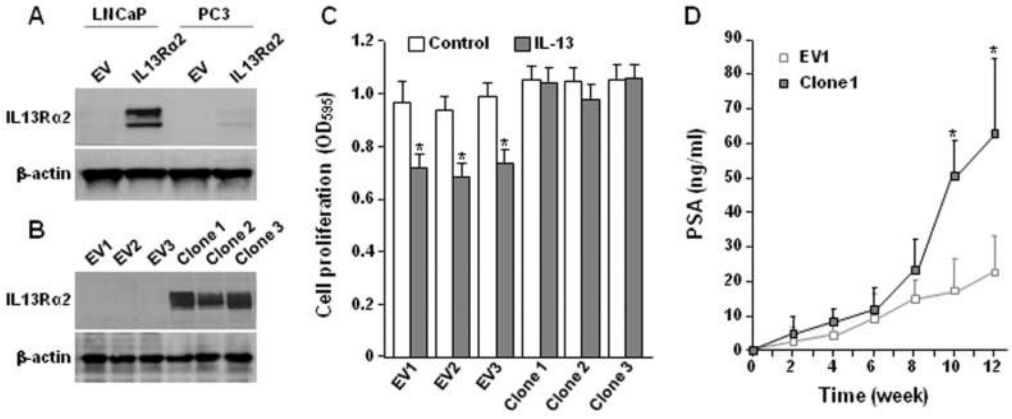

Figure 4. IL13Rα2 expression renders LNCaP cells insensitive to IL-13 treatment

A, in the upper panel, LNCaP and PC-3 prostate cancer cells were transfected with the empty vector (EV) or the IL13Rα2 expression construct in the same vector (IL13Rα2) for 48 hours, and were subjected to western blotting for IL13Rα2 with the MAB614 antibody. In the lower panel, β-actin was used to show equal loading. The results are representative of three separate experiments. **B**, in the upper panel, LNCaP clones transfected with the empty vector (EV1, EV2, and EV3) and clones transfected with the IL13Rα2 expression construct (Clone 1, Clone 2, and Clone 3) were subjected to western blotting for IL13Rα2. In the lower panel, β-actin was used to show equal loading. **C**, the LNCaP clones were subjected to IL-13 treatment (25 ng/ml) for 72 hours in cell proliferation assay. The results are representative of two separate experiments. For each clone, an asterisk indicates statistical significance ($P < 0.05$) compared to the control. **D**, IL13Rα2 promotes xenograft tumor formation. A representative LNCaP clone (Clone 1) expressing IL13Rα2 was used for intratibial tumor formation in athymic mice. A clone transfected with the vector (EV1) was used for comparison. PSA concentration in the blood was used to reflect tumor growth. Each data point represents the mean of PSA measurements from six mice. An asterisk indicates statistical significance ($P < 0.05$) compared to control group at the same time point.

Table 1

Primers used for RT-PCR analyses and expected product sizes.

Gene	GenBank Accession No	Primers (forward) (Reverse)	Expected size (bp)
IL-4	NM_000589	ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTC TCAGCTCGAACACTTTGAATATTTCTCTCTCATG	462
IL-13	NM_002188	ATGCATCCGCTCCTCAATCCTCTCCTGTTG TCAGTTGAACTGTCCCTCGCGAAAAAGTTTC	441
IL13Rα1	NM_001560	CTCCGAGGCGAGAGGCTGCATGGAGTG TCACTGAGAGGCTTTCTTCAGGTTTTCTATCAG	1,284
IL13Rα2	NM_000640	ATGGCTTTCGTTTGCTTGGCTATCGGATGCTTATATAC TCATGTATCACAGAAAAATTCTGGAATCATTTTTG	1,143
IL4Rα	NM_000418	GTTTGGGGCTAGCAGTGGGGAAGAGGGGTATAAG TAAGAGACCCTCATGTATGTGGGTCCCACGGAGAC	615
IL2Rγc	NM_000206	ATGTTGAAGCCATCATTACCATTCACATC TCAGGTTTCAGGCTTTAGGGTGTAACATG	1,110
GAPDH	NM_002046	ATGGGGAAGGTGAAGGTCGGAGTCAAC TTACTCCTTGGAGGCCATGTGGGCCATGAG	1,008