Retinoid X receptor suppresses a metastasis-promoting transcriptional program in myeloid cells via a ligand-insensitive mechanism

Mate Kiss[a], Zsolt Czimmerer[a,b], Gergely Nagy[b], Pawel Bieniasz-Krzywiec[c,d], Manuel Ehling[c,d], Attila Pap[a], Szilard Poliska[a,e], Pal Boto[f], Petros Tzerpos[e], Attila Horvath[a], Zsuzsanna Kolostyak[a], Bence Daniel[g], Istvan Szatmari[f], Massimiliano Mazzone[c,d], and Laszlo Nagy[a,b,g,1]

[a]Nuclear Receptor Research Laboratory, Department of Biochemistry and Molecular Biology, University of Debrecen, 4032 Debrecen, Hungary; [b]MTA-DE "Lendület" Immunogenomics Research Group, University of Debrecen, 4032 Debrecen, Hungary; [c]Laboratory of Tumor Inflammation and Angiogenesis, Center for Cancer Biology, VIB, 3000 Leuven, Belgium; [d]Laboratory of Tumor Inflammation and Angiogenesis, Department of Oncology, KU Leuven, 3000 Leuven, Belgium; [e]UD-GenoMed Ltd., 4032 Debrecen, Hungary; [f]Stem Cell Differentiation Laboratory, Department of Biochemistry and Molecular Biology, University of Debrecen, 4032 Debrecen, Hungary; and [g]Genomic Control of Metabolism Program, Sanford Burnham Prebys Medical Discovery Institute at Lake Nona, Orlando, FL 32827

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Retinoid X receptor (RXR) regulates several key functions in myeloid cells, including inflammatory responses, phagocytosis, chemokine secretion, and proangiogenic activity. Its importance, however, in tumor-associated myeloid cells is unknown. In this study, we demonstrate that deletion of RXR in myeloid cells enhances lung metastasis formation while not affecting primary tumor growth. We show that RXR deficiency leads to transcriptomic changes in the lung myeloid compartment characterized by increased expression of prometastatic genes, including important determinants of premetastatic niche formation. Accordingly, RXR-deficient myeloid cells are more efficient in promoting cancer cell migration and invasion. Our results suggest that the repressive activity of RXR on prometastatic genes is mediated primarily through direct DNA binding of the receptor along with nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) corepressors and is largely unresponsive to ligand activation. In addition, we found that expression and transcriptional activity of RXRα is down-modulated in peripheral blood mononuclear cells of patients with lung cancer, particularly in advanced and metastatic disease. Overall, our results identify RXR as a regulator in the myeloid cell-assisted metastatic process and establish lipid-sensing nuclear receptors in the microenvironmental regulation of tumor progression.

retinoid X receptor | metastasis | premetastatic niche | myeloid cell | NCoR

Retinoid X receptor (RXR) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors that bind various lipophilic hormones and lipid metabolites. Sensing the lipid milieu by RXR, in concert with other nuclear receptors, plays critical roles in physiology, including the regulation of organogenesis, lipid metabolism, and skin homeostasis (1). RXR is able to bind vitamin A derivatives 9-cis-retinoic acid and 9-cis-13,14-dihydroretinoic acid as well as fatty acids such as docosahexanoic acid and phytanic acid (1, 2). The receptor has three isotypes, RXRα, RXRβ, and RXRγ, which appear to be functionally interchangeable in tissues in which more than one isotype is expressed (3). RXR has a unique integrative function in nuclear receptor signaling because of its ability to form homodimers as well as heterodimers with several other members of the superfamily, such as PPAR, LXR, RAR, and VDR. RXR and its heterodimers have a profound effect on the function of myeloid cells by linking cellular metabolism and immune function (4, 5). Specific activation of RXR has been shown to up-regulate chemokine expression, promote phagocytosis of apoptotic cells, and attenuate antiviral responses in myeloid cells (6–8). To further delineate the function of the receptor, we recently mapped the genomic binding sites of RXR in macrophages and identified a network of RXR-bound enhancers that control angiogenic genes including *Vegfa*, *Hbegf*, *Litaf*, and *Hipk2* (9). Activation of these enhancers by RXR induced a proangiogenic transcriptional program and phenotype (9). Interestingly, we found that half of the 5,200 RXR-occupied genomic sites were transcriptionally inactive in macrophages (9). This suggested that the receptor may also possess extensive regulatory functions in myeloid cells that are distinct from ligand recognition and activation. These observations provided a rationale to investigate whether RXR influences tumor progression through regulating the phenotype of recruited or tissue-resident myeloid cells.

Results

RXR Deletion in Myeloid Cells Promotes Metastasis to the Lung. In myeloid cells, RXRα shows the highest expression and RXRβ is expressed at a lower level, while RXRγ is not expressed (5). Specific deletion of RXRα in myeloid cells was achieved by crossing mice bearing lox-P-targeted RXRα ($RXR\alpha^{fl/fl}$) with mice carrying the lysozyme-M Cre (LysM-Cre) recombinase transgene. To exclude the potential compensatory effects of RXRβ in the absence of RXRα resulting from the functional

Significance

Metastasis formation from malignant tumors is the leading cause of cancer-related deaths. There is an increasing body of evidence indicating that immune cells in distant organs actively contribute to this process by establishing a tissue environment that is hospitable for cancer cells. In this study, we show that deletion of retinoid X receptor (RXR), a cellular sensor of vitamin A metabolites, specifically in the myeloid lineage of the immune system, leads to an enhanced metastasis rate. We also demonstrate that RXR inhibits the expression of a number of genes that encode proteins involved in the promotion of metastasis formation. Surprisingly, our results suggest that this activity of RXR is independent of the presence of its activators.

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[1]To whom correspondence should be addressed. Email: lnagy@sbpdiscovery.org.

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redundancy of RXR isotypes (3, 10), we crossed LysM-Cre RXRα$^{fl/fl}$ with RXRβ$^{-/-}$ mice. Given the low fertility and viability of the resulting LysM-Cre RXRα$^{fl/fl}$ RXRβ$^{-/-}$ mice (11), we transplanted their bone marrow to syngeneic C57BL/6J mice. This way, we were able to generate age-matched groups of mice bearing LysM-Cre RXRα$^{fl/fl}$ RXRβ$^{-/-}$ and LysM-Cre RXRα$^{+/+}$ RXRβ$^{-/-}$ bone marrow (hereafter referred to as RXRα$^{fl/fl}$ and RXRα$^{+/+}$, respectively).

To investigate the effect of RXRα deletion in the myeloid infiltrate on tumor progression, we s.c. implanted RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice with Lewis lung carcinoma (LLC), a murine model of non-small cell lung carcinoma showing abundant infiltration of myeloid cells from early stages. Primary tumor growth remained unaltered (Fig. 1*A*), and the immune cell infiltration in RXRα$^{fl/fl}$ mice was largely unaltered with a significantly lower abundance of M1-like CD11b^{+}Ly6G^{-}Ly6C^{-}MHC-II^{high} macrophages (*SI Appendix*, Fig. S1). However, the frequency of M2-like MHC-II^{low} macrophages and other immune cell populations was not affected by RXRα deficiency (*SI Appendix*, Fig. S1). Although primary tumor growth was not affected, we observed a higher frequency of animals with spontaneous lung metastases, as well as a higher number of metastatic nodules in the RXRα knock-outs compared with controls (Fig. 1 *B* and *C*). Thus, we further investigated the role of myeloid cell-expressed RXRα in lung colonization by using an experimental metastasis model. Consistent with the results obtained in the LLC model, we found that B16-F10 melanoma cells formed a higher number of macroscopic and microscopic metastases in the lungs of RXRα$^{fl/fl}$ mice after i.v. injection (Fig. 2 *A*–*C*). RXRα$^{fl/fl}$ lungs contained a considerably higher frequency of micrometastases with diameters less than 100 μm, demonstrating that extravasation followed by micrometastasis formation in the lung is more efficient in the presence of RXRα-deficient myeloid cells (Fig. 2*D*). Since survival and proliferation of extravasated cancer cells at the distant organ is a critical early step of metastasis, we also examined the effect of myeloid cell RXRα on the apoptotic and mitotic rate of metastatic cancer cells. Staining of B16-F10 lung metastases with Ki67, a marker of cell proliferation, did not show major differences between RXRα$^{fl/fl}$ and RXRα$^{+/+}$ lungs (Fig. 2*E*). The apoptosis rate, as assessed by staining of cleaved caspase-3, was not significantly different in metastases of RXRα$^{fl/fl}$ lungs either (Fig. 2*E*). This suggested that myeloid-specific RXRα deficiency does not affect the proliferation or survival of metastatic cancer cells in the lung, and the enhanced metastasis rate in RXRα$^{fl/fl}$ mice originates principally from enhanced lung colonization. Altogether, these results indicate that deletion of RXRα in the myeloid compartment generates a lung microenvironment that is more permissive toward metastasis formation.

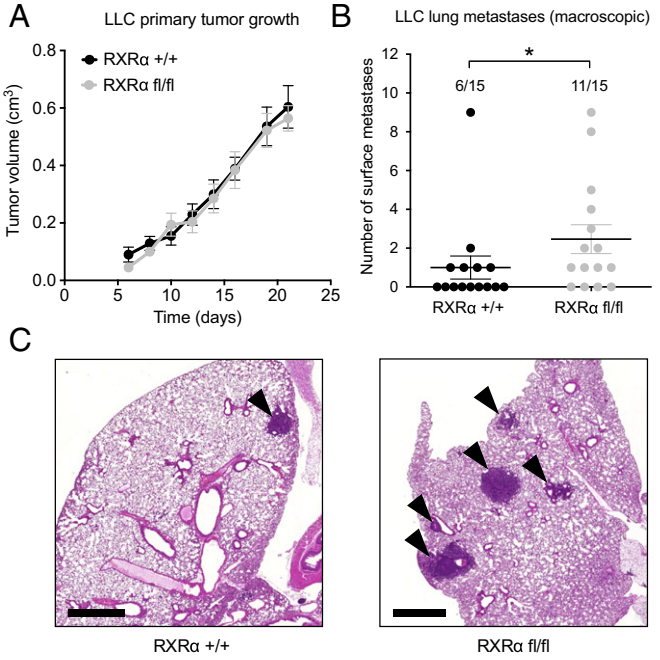


Fig. 1. RXRα deletion in myeloid cells enhances Lewis lung carcinoma metastasis. (*A*) Subcutaneous LLC tumor growth in mice with myeloid cell-specific deletion of RXRα (RXRα$^{fl/fl}$) and control (RXRα$^{+/+}$) mice (n = 5–7/group). (*B*) Number of macroscopic lung metastases in RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice (*P < 0.05, Mann-Whitney test, n = 15/group, data combine two independent experiments). (*C*) Representative images of hematoxylin and eosin-stained midcoronal lung sections from RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice. Arrowheads indicate metastatic nodules. (Scale bar, 1 mm.) Graphs on *A* and *B* show mean ± SEM.

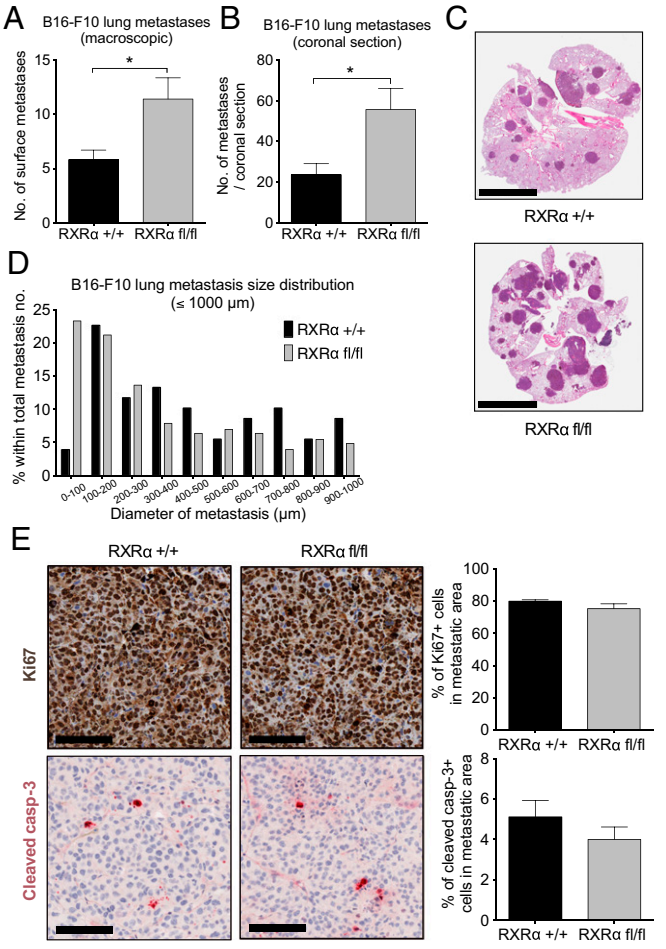


Fig. 2. RXRα deletion in myeloid cells results in enhanced lung colonization by B16-F10 melanoma cells. (*A*) Number of macroscopic lung metastases in RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice (*P < 0.05, unpaired Student's t test, n = 10–13/group, data combine two independent experiments). (*B*) Number of microscopic lung metastases on midcoronal lung sections in RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice (*P < 0.05, unpaired Student's t test, n = 8/group). (*C*) Representative images of hematoxylin and eosin-stained midcoronal lung sections from RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice. (Scale bar, 6 mm.) (*D*) Size distribution of B16-F10 melanoma lung metastases with a diameter ≤1,000 μm in RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice. Metastasis diameter data are pooled from eight lungs/group. (*E*) Representative images and quantification of Ki67 and cleaved caspase-3 stainings on midcoronal lung sections with B16-F10 melanoma metastases from RXRα$^{fl/fl}$ and RXRα$^{+/+}$ mice (Scale bar, 100 μm.) (P > 0.05, unpaired Student's t test, n = 5/group). Bar graphs on *A*, *B*, and *E* show mean ± SEM.

RXR Represses a Prometastatic Gene Set in Pulmonary Myeloid Cells. Our results showing enhanced lung colonization of cancer cells in mice with myeloid-specific RXRα deficiency prompted us to investigate whether the absence of RXRα has any effect on the abundance or phenotype of pulmonary myeloid cells. Flow cytometry analysis of the pulmonary myeloid compartment revealed that the lack of RXRα was associated with slightly decreased numbers of $CD45^+CD11b^+F4/80^+Ly6G^-$ lung macrophages in naive mice (Fig. 3*A*). In contrast, neither the resident population nor the metastasis-induced recruitment of $CD45^+CD11b^+F4/80^-Ly6G^+$ neutrophils was affected by the absence of myeloid RXRα (Fig. 3*A*). Along with metastasis-associated macrophages differentiated from inflammatory monocytes, lung-resident macrophages have recently been reported to promote the formation of lung metastases (12). Accordingly, LysM-Cre-mediated deletion of an RXR heterodimeric partner PPARγ in myeloid cells results in a moderate decrease of alveolar macrophages (13), and these mice develop fewer pulmonary metastases (14). For these reasons, we assumed that the increase in metastasis formation cannot be explained by the decreased number of lung-resident macrophages in $RXR\alpha^{fl/fl}$ mice. Thus, we decided to investigate whether the lack of RXRα can alter the phenotype of pulmonary myeloid cells, resulting in a more permissive tissue environment for metastatic cancer cells. To characterize the cellular consequences of RXRα deletion in myeloid cells, we performed transcriptomic analysis via RNA sequencing on the $CD45.2^+CD11b^+$ myeloid compartment isolated from naive lungs of $RXR\alpha^{+/+}$ and $RXR\alpha^{fl/fl}$ mice. Expression of the CD45.2 pan-leukocyte antigen by $CD11b^+$ myeloid cells confirmed their transplanted bone marrow origin. We found 1,284 differentially expressed genes (adjusted P value < 0.01) comparing $RXR\alpha^{+/+}$ and $RXR\alpha^{fl/fl}$ cells (Fig. 3*B* and Dataset S1). Interestingly, gene ontology analysis identified "regulation of cell migration" and "positive regulation of cell migration" along with "cytokine-mediated signaling pathway" as the most significantly enriched biological processes among the genes up-regulated in RXRα-deficient pulmonary myeloid cells (Fig. 3*C*). Using Ingenuity Pathway Analysis as an alternative approach yielded similar results (*SI Appendix*, Table S1). Accordingly, within the RXRα-repressed gene set, we could identify a number of genes encoding secreted factors that have been previously described to promote metastasis, including inflammatory mediators (*C1qa*, *Il1a*, *Il1b*, *Tnf*, *Lcn2*), growth factors (*Gas6*, *Hbegf*, *Igf1*, *Pdgfa*, *Pdgfb*), extracellular matrix proteins (*Ecm1*, *Vcan*), matrix-degrading proteases (*Mmp2*, *Ctsb*, *Ctss*), proinflammatory S100 calcium-binding proteins (*S100a4*, *S100a8*), and the proinvasive secreted semaphorin, *Sema4d* (Fig. 3*D* and *SI Appendix*, Table S2). In addition, upstream regulator analysis of differentially expressed genes in RXRα-deficient myeloid cells predicted the activation of several inflammatory pathways including TNF, TLR2, and TLR4, which have a crucial role in initiating an inflammatory microenvironment in the lung, thereby promoting metastatic colonization (15, 16) (*SI Appendix*, Table S3).

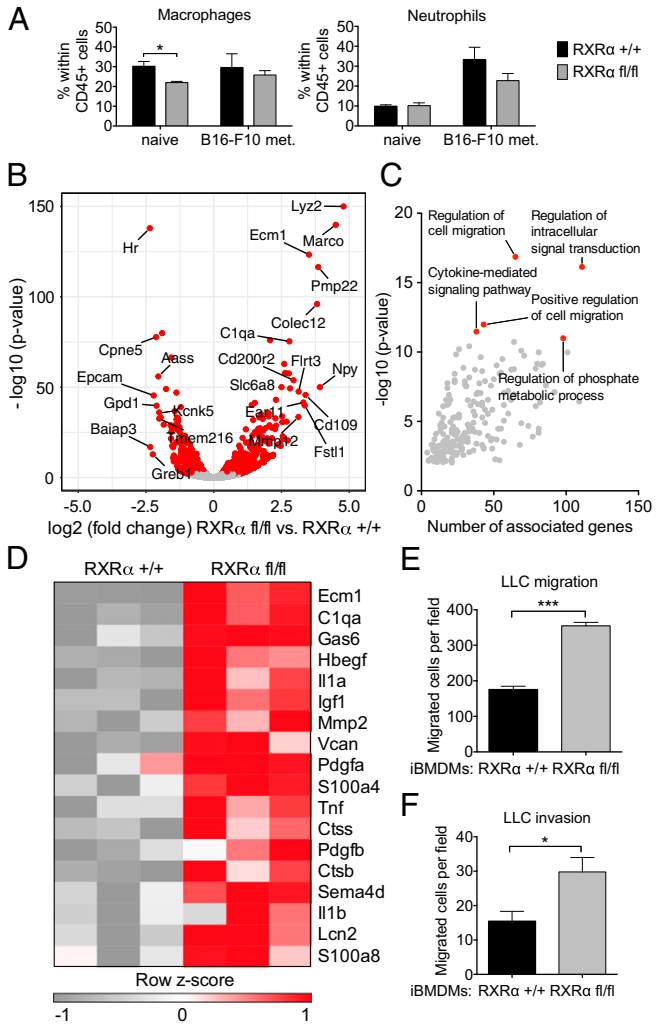


Fig. 3. RXRα represses a prometastatic gene set in pulmonary myeloid cells. (*A*) Relative numbers of myeloid cells in the lungs of $RXR\alpha^{+/+}$ and $RXR\alpha^{fl/fl}$ mice in the presence or absence of B16-F10 melanoma metastases (*P < 0.05, unpaired Student's t test, n = 3/group). (*B*) Volcano plot showing transcriptomic differences in $CD45^+CD11b^+$ pulmonary myeloid cells from $RXR\alpha^{fl/fl}$ mice compared with $RXR\alpha^{+/+}$. Red dots represent differentially expressed genes (adjusted P < 0.01, Wald test, n = 3/group). (*C*) Dot plot showing significantly enriched gene ontology biological processes within the up-regulated genes in $RXR\alpha^{fl/fl}$ pulmonary myeloid cells. The top five enriched GO terms by P value are highlighted. (*D*) Heat map showing expression changes of selected prometastatic genes in $RXR\alpha^{+/+}$ and $RXR\alpha^{fl/fl}$ pulmonary myeloid cells. (*E*) Number of migrated LLC cells in the presence of $RXR\alpha^{+/+}$ or $RXR\alpha^{fl/fl}$ iBMDMs in Transwell migration assay. The experimental setup is shown in *SI Appendix*, Fig. S2*A* (***P < 0.001, unpaired Student's t test, n = 3/group). (*F*) Number of migrated LLC cells through Matrigel layer in the presence of $RXR\alpha^{+/+}$ or $RXR\alpha^{fl/fl}$ iBMDMs in Transwell invasion assay. The experimental setup is shown in *SI Appendix*, Fig. S2*B* (*P < 0.05, unpaired Student's t test, n = 3/group). Bar graphs on *A*, *E*, and *F* show mean ± SEM.

These results strongly suggested that RXRα constitutively represses a large set of genes involved in the positive regulation of cell migration and metastasis formation. This led us to hypothesize that the relief of repression (de-repression) of these genes in the absence of RXRα results in a migration-promoting phenotype in myeloid cells. To test this hypothesis, we used $RXR\alpha^{fl/fl}$ and $RXR\alpha^{+/+}$ immortalized bone marrow-derived macrophages (iBMDM). As expected, based on the migration-promoting transcriptomic signature induced in the absence of RXRα, $RXR\alpha^{fl/fl}$ iBMDMs were significantly more efficient in promoting the migration of LLC cells compared with $RXR\alpha^{+/+}$ cells (Fig. 3*E* and *SI Appendix*, Fig. S2*A*). Interestingly, RXRα deficiency increased not only the migration-promoting activity of iBMDMs but also their proinvasive effect. LLC cells were more efficient in invading through a Matrigel extracellular matrix layer in the presence of RXRα-deficient iBMDMs (Fig. 3*F* and *SI Appendix*, Fig. S2*B*). While enhancing cancer cell migration and invasion, RXRα-deficient iBMDMs did not promote cancer cell proliferation (*SI Appendix*, Fig. S2*C*). Overall, these results indicate that the transcriptomic signature generated by RXRα-deficiency in myeloid cells translates to a metastasis-promoting cellular phenotype.

RXR-Mediated Repression of Prometastatic Genes Is Predominantly Ligand-Insensitive. Next, we sought to determine whether the RXR-mediated repression in myeloid cells can be enhanced or suspended by the presence of an RXR-activating ligand. To

answer this, we used our previously published RNA-seq data from BMDMs treated with a highly selective, synthetic RXR agonist LG268 (9). The short-term ligand activation (30/60/120 min) enabled us to exclude secondary transcriptional changes after activation of the receptor. By examining the ligand response of RXR-repressed genes identified in pulmonary myeloid cells, we found that only a small fraction of them (14.1%) showed gene expression change in response to RXR activation (8/2, 29/3, 58/25 up-/down-regulated from 695 after 30, 60, 120 min of LG268 stimulation, respectively) (Fig. 4*A*). In the previously described prometastatic gene set, only *Hbegf*, *Pdgfa*, and *Pdgfb* showed significant up-regulation on ligand stimulus (Fig. 4*B*). This suggested that the majority of RXR-repressed genes in myeloid cells (including genes with prometastatic effect) cannot be modulated by RXR agonists. This was further supported by the observation that the cancer cell migration-promoting activity of iBMDMs was not altered after pretreatment with selective RXR agonist LG268 (Fig. 4*C*).

RXR Colocalizes with NCoR/SMRT Corepressors to Mediate Repression of Prometastatic Genes. Unliganded RXR homodimers and heterodimers bind the promoters and/or enhancers of their target genes in association with corepressors nuclear receptor corepressor (NCoR) or silencing mediator of retinoic acid and thyroid hormone receptor (SMRT). Using our previously published RXR ChIP-seq dataset from BMDMs (9), we determined which genes show RXR binding in the proximity of their transcription start site (±100 kb) at the genome-wide level. Intriguingly, gene ontology analysis of these RXR-bound genes revealed a significant overrepresentation of genes involved in the regulation of cell migration and activation of the immune response (*SI Appendix*, Fig. S3). This suggested that the major genomic targets of RXR binding (and possibly repression) in the steady state are genes that can be functionally linked to cell migration and inflammation. Next, we focused on genes that are repressed by RXR in pulmonary myeloid cells and examined whether RXR binding sites can be found in the vicinity of them. We could detect RXR binding sites within 100 kb of the transcription start sites in 66% of RXR-repressed genes. This suggested that direct DNA binding by the receptor accounts for its repressive effect by the majority of repressed genes. As mentioned earlier, NCoR and SMRT corepressors are the most common interacting partners of RXR in mediating transcriptional repression. To determine whether RXR binding is associated with NCoR or SMRT binding at RXR-repressed genes, we combined our RXR ChIP-seq data with public NCoR and SMRT ChIP-seq datasets from BMDMs (17). Interestingly, 59% of RXR binding sites detected in the vicinity of RXR-repressed genes showed overlap with both NCoR and SMRT, while 12% and 7% showed overlap with only NCoR or only SMRT, respectively. In addition, NCoR and SMRT occupancy in the proximity of RXR-repressed genes was higher at genomic sites where RXR was also present, suggesting that the presence of RXR facilitates the binding of these corepressors (Fig. 5*A*). When we examined the genomic loci of the members of RXR-repressed prometastatic gene set identified in pulmonary myeloid cells, we detected overlapping RXR/NCoR/SMRT binding in the close proximity of 11 of the 18 genes (Fig. 5*B*). It has been shown previously that nuclear receptor-mediated transcriptional repression largely required either NCoR alone or both NCoR and SMRT, only a small fraction of genes showing repression specifically by SMRT (18). Accordingly, deletion of the dominant corepressor NCoR in iBMDMs resulted in the up-regulation of *Il1a*, *Il1b*, *Pdgfb*, *Sema4d*, *Igf1*, and *Ctss*, confirming the requirement of NCoR for the repression of these genes (Fig. 5*C*). Lack of de-repression by several RXR/NCoR/SMRT-bound genes in NCoR-deficient iBMDMs suggested the existence of compensatory mechanisms emerging in the absence of NCoR, as reported previously (19).

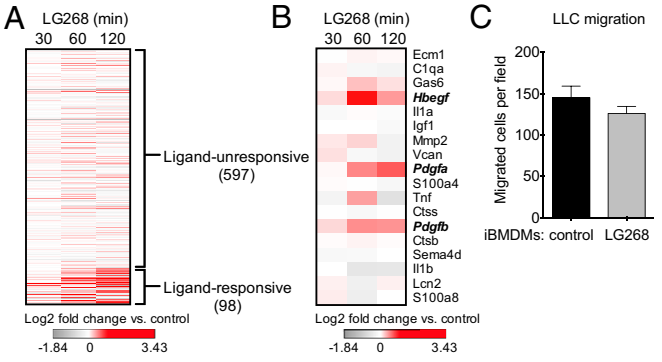


Fig. 4. RXR-mediated repression of prometastatic genes is predominantly ligand-insensitive. (*A*) Heat map showing expression changes in response to synthetic RXR agonist LG268 in BMDMs among RXR-repressed genes identified in pulmonary myeloid cells (adjusted $P < 0.01$, Wald test, $n = 2$/time point). (*B*) Heat map showing expression changes in response to synthetic RXR agonist LG268 in BMDMs among selected RXR-repressed prometastatic genes identified in pulmonary myeloid cells. Genes with significant expression changes are shown in bold italics (adjusted $P < 0.01$, Wald test, $n = 2$/group). (*C*) Number of migrated LLC cells in the presence of control or LG268-pretreated iBMDMs in Transwell migration assay ($P > 0.05$, unpaired Student's *t* test, $n = 3$/group).

RXR Signaling Is Down-Modulated in Peripheral Blood Mononuclear Cells of Patients with Non-Small Cell Lung Cancer. Finally, we set out to determine whether any alterations in RXR signaling can be observed in patients with cancer. Deletion of RXRα in mice results in embryonic lethality caused by abnormal development of the heart (1). Similarly, DNA sequence data from the Exome Aggregation Consortium showed that loss-of-function mutations in genes coding any of the RXR isotypes are highly improbable, with a probability of loss-of-function intolerance of 94%, 100%, and 94% for RXRα, RXRβ, and RXRγ, respectively (20). Thus, since loss-of-function mutations of RXR are predicted to be very rare, we aimed to investigate whether any changes in RXR expression can be observed in myeloid cells of patients with cancer that could potentially influence its repressive activity. As expression data from tissue-resident or metastasis-associated myeloid cells is difficult to obtain in the case of patients with cancer, we decided to analyze gene expression changes in peripheral blood mononuclear cells (PBMCs). PBMCs have been described to exhibit distinct gene expression changes in a number of malignancies (21–23). We chose to analyze the gene expression of PBMCs from patients with non-small cell lung cancer (NSCLC) and cancer-free control patients, using the largest publicly available dataset including samples from 137 patients with NSCLC and 91 cancer-free control patients (22). Intriguingly, we found that PBMCs from patients with NSCLC showed a significantly lower expression of RXRα (Fig. 6*A*). In addition, the mean expression level of RXRα exhibited decreasing kinetics during disease progression, showing the lowest expression in patients with advanced and metastatic disease (stage III-IV) (Fig. 6*A*). RXRβ and RXRγ isotypes displayed lower average expression than RXRα in PBMCs and did not show any changes in patients with NSCLC (Fig. 6*A*). These data showed that RXRα, the dominant RXR isotype expressed in myeloid cells, was down-regulated in individuals with NSCLC, and its decreased expression was not compensated for by the up-regulation of other RXR isotypes. Next, we wanted to determine whether down-regulation of RXRα in patients with lung cancer is associated with its altered transcriptional activity. RXR's activity can be assessed by examining the regulation of the target genes of its

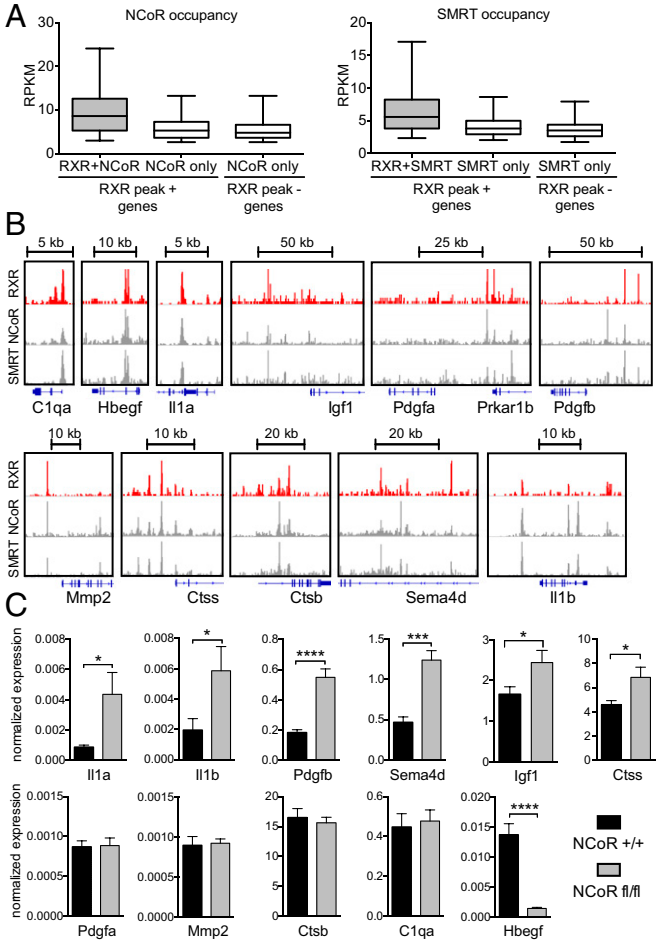
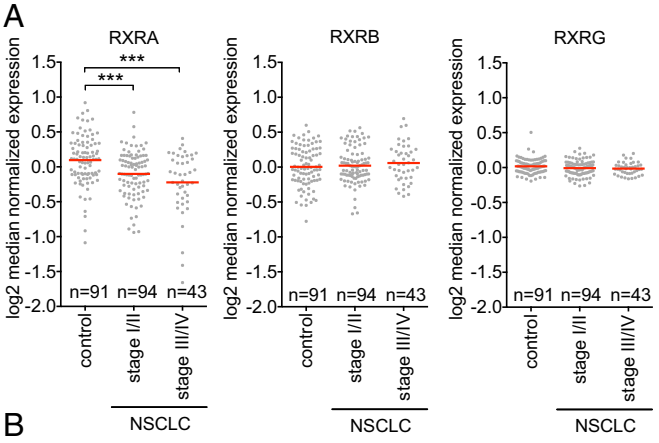


Fig. 5. RXR colocalizes with NCOR/SMRT corepressors to mediate repression of prometastatic genes. (*A*) NCoR and SMRT occupancy in BMDMs at the genomic sites of RXR-repressed genes identified in pulmonary myeloid cells with/without overlap with RXR (box plots show median and 5–95 percentiles). (*B*) Genomic loci of selected prometastatic genes showing overlapping RXR/NCoR/SMRT ChIP-seq peaks in BMDMs. (*C*) Expression of selected prometastatic genes in $NCoR^{+/+}$ and $NCoR^{fl/fl}$ iBMDMs (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, unpaired Student's *t* test, $n = 7$/group).

heterodimeric partners, since specific target genes for homodimeric RXR signaling have not been unequivocally established. To identify transcriptional regulators that show altered activity in NSCLC, we determined the differentially expressed genes between PBMCs of patients with NSCLC and cancer-free control patients and performed an upstream regulator prediction on these genes using Ingenuity Pathway Analysis. Interestingly, among the top 10 upstream regulators whose activity was predicted to be strongly inhibited in patients with NSCLC, we found two nuclear receptors, PPARγ and LXRα, that require RXR as a heterodimeric partner for their transcriptional activity (Fig. 6*B*). The inhibited transcriptional activity of PPARγ/RXR and LXRα/RXR heterodimers in patients with NSCLC could not be explained by the down-regulated expression of PPARγ and LXRα, as their expression remained unaltered (*SI Appendix*, Fig. S4). Overall, these data suggest that the expression and transcriptional activity of RXRα is down-modulated in the immune system of patients with NSCLC, particularly in advanced and metastatic disease.

Discussion

Our study demonstrates that RXRα expressed by myeloid cells in the lung is essential to maintain a metastasis-resistant tissue microenvironment. Our data suggest that steady-state transcriptional repression by RXR, partly in concert with NCoR and SMRT corepressors in myeloid cells, controls the expression of several genes whose protein products facilitate the metastatic spread of cancer cells in distant organs. RXR appears to be unique in its ability to form homodimers, as well as heterodimers, with a diverse range of nuclear receptors. Therefore, it is possible that prometastatic genes in pulmonary myeloid cells are repressed both by RXR homodimers and heterodimers. However, the relative contribution of homo- and heterodimers is difficult to determine, since they show redundancy in their binding site preferences, and target genes regulated exclusively by RXR homodimers have not been identified yet (24). The absence of RXR-mediated repression in myeloid cells leads to elevated expression of prometastatic regulators, creating a tissue microenvironment favoring cancer cell extravasation and micrometastasis formation. This process shares similarities to the formation of the premetastatic niche, a tumor-induced receptive and supportive tissue microenvironment that supports cancer cell colonization and survival in a secondary organ site (25, 26). Interestingly, several RXR-repressed genes identified in our work have been described as important regulators involved in the formation of premetastatic niches. We found that RXR represses both *S100a4* and *S100a8* in pulmonary myeloid cells. The protein products of these genes act as central regulators during the course of premetastatic niche formation by generating an inflammatory microenvironment both directly and indirectly via the up-regulation of serum amyloid A3, resulting in enhanced cancer cell adherence and colonization (25, 26). In addition, we found that $RXR\alpha^{fl/fl}$ pulmonary myeloid cells up-regulated TNFα, a cytokine that has been identified as a key component of the inflammatory milieu in the premetastatic niche in various tissue environments



Rank	Upstream Regulator	Activation z-score	p-value of overlap
1.	LIPE	-2.621	0.00305
2.	CBFB	-2.333	0.0196
3.	***PPARG***	***-2.142***	***0.0246***
4.	PPP2R5C	-2	0.0161
5.	CD3	-1.992	0.0000153
6.	***NR1H3 (LXRα)***	***-1.987***	***0.0378***
7.	FGF7	-1.962	0.0411
8.	CD28	-1.734	0.00101
9.	STAP2	-1.732	0.00736
10.	PSEN1	-1.687	0.0406

Fig. 6. RXR signaling is down-modulated in PBMCs of patients with NSCLC. (*A*) Expression of RXR isotypes in PBMCs of patients with NSCLC and cancer-free control patients. (Graphs show mean. ***adjusted $P < 0.001$, Mann-Whitney test) (*B*) Top 10 upstream regulators by *z*-score predicted to be inhibited in PBMCs of patients with NSCLC compared with cancer-free control patients ($P < 0.05$). RXR heterodimeric partners are shown in bold italics.

(15, 25, 26). Similarly, versican, an extracellular matrix proteoglycan with multifaceted roles in premetastatic niche formation, was also up-regulated in RXRα$^{fl/fl}$ pulmonary myeloid cells (25, 26). In the Lewis lung carcinoma model, tumor-derived versican activated myeloid cells through TLR2 to induce an inflammatory microenvironment in the premetastatic lung (15). In contrast, in the MMTV-PyMT model of breast carcinoma, versican secreted by CD11b^{+}Ly6C^{high} myeloid cells in premetastatic lungs stimulated mesenchymal to epithelial transition of cancer cells, leading to enhanced metastatic colonization (27). Another characteristic feature of premetastatic niche is tissue remodeling and degradation of the extracellular matrix that favors extravasation of circulating cancer cells (25, 26). Analogously, RXRα$^{fl/fl}$ myeloid cells in the lung up-regulated several proteases capable of matrix remodeling, including MMP2, cathepsin B, and cathepsin S. As a consequence, RXR-deficient iBMDMs had an enhanced capacity to promote cancer cell invasion through an extracellular matrix layer.

Administration of selective RXR agonists have a well-established beneficial effect in solid tumors because of their capacity to induce differentiation and/or apoptosis of cancer cells (28). Nevertheless, the influence of RXR modulation both on the stromal compartment of primary tumor microenvironment and on premetastatic niches remains to be determined. Our findings suggest that the majority of genes differentially expressed in RXRα-deficient pulmonary myeloid cells do not respond to RXR activation. Therefore, it appears unlikely that endogenous or synthetic RXR agonists can alter the repressive activity of the receptor on prometastatic regulators. To therapeutically exploit RXR's ability to control the expression of several key regulators in metastasis, RXR-specific inverse agonists could be used. Inverse agonists have the ability to stabilize the nuclear receptor–corepressor complex and enhance transcriptional repression (29). Although RXR-specific inverse agonists are not yet available, similar compounds have already been developed and used successfully in preclinical studies for several other nuclear receptors including RAR, LXR, and ROR (30–33). This class of RXR-specific ligands could enhance the repressive effect of RXR on prometastatic regulators, potentially rendering the lung microenvironment less permissive toward metastatic colonization.

Materials and Methods

Details of mice and bone marrow transplantation, cell lines and tumor models, histology, flow cytometry and cell sorting, RNA-seq, ChIP-seq analysis, immortalization of mouse bone marrow-derived macrophages, cancer cell migration and invasion assays, real-time quantitative PCR, microarray analysis, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay, and statistical analysis are described in the *SI Appendix*. All experiments were carried out in accordance with the institutional ethical guidelines at the University of Debrecen (license no.: 21/2011/DE MAAB), following the European regulations.

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