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ORIGINAL ARTICLE

MiR-27b targets *PPARγ* to inhibit growth, tumor progression and the inflammatory response in neuroblastoma cells

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The peroxisome proliferators-activated receptor (*PPAR)*γ pathway is involved in cancer, but it appears to have both tumor suppressor and oncogenic functions. In neuroblastoma cells, miR-27b targets the 3′ untranslated region of *PPARγ* and inhibits its mRNA and protein expression. miR-27b overexpression or *PPARγ* inhibition blocks cell growth *in vitro* and tumor growth in mouse xenografts. *PPARγ* activates expression of the pH regulator *NHE1*, which is associated with tumor progression. Lastly, miR-27b through *PPARγ* regulates nuclear factor-κB activity and transcription of inflammatory target genes. Thus, in neuroblastoma, miR-27b functions as a tumor suppressor by inhibiting the tumor-promoting function of *PPARγ*, which triggers an increased inflammatory response. In contrast, in breast cancer cells, *PPARγ* inhibits *NHE1* expression and the inflammatory response, and it functions as a tumor suppressor. We suggest that the ability of *PPARγ* to promote or suppress tumor formation is linked to cell type-specific differences in regulation of *NHE1* and other target genes.

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Introduction

Peroxisome proliferators-activated receptors (PPAR) are members of the nuclear receptor superfamily of ligand-activated transcription factors. Three isoforms, PPARα, PPARβ/δ and PPARγ, are encoded by three genes that respond to diverse, but distinct, sets of ligands (Michalik *et al*., 2004). PPARγ has emerged as an attractive target for cancer therapy because of its association with many human cancers such as colon, thyroid, breast and prostate (Michalik *et al*., 2004). PPARγ is abundant in adipose tissues and is also expressed at a lower level in the skeletal muscles, liver, heart, intestine, vascular smooth muscle, lung, breast, colon and prostate. Interestingly abundant PPARγ expression has been detected in different tumors such as transformed human B lymphocyte and myeloid cells lines, astrocytomas (Chattopadhyay *et al*., 2000), glioblastoma (Nwankwo and Robbins, 2001; Morosetti *et al*., 2004) and neuroblastoma (Han *et al*., 2001).

The role of PPARγ in tumor development is controversial. It has been suggested that PPARγ is a tumor suppressor, because ligands that activate PPARγ promote growth inhibition and apoptosis in cancers of breast (Mueller *et al*., 1998; Mehta *et al*., 2000; Kim *et al*., 2006), colon (Sarraf *et al*., 1998), liposarcoma (Tontonoz *et al*., 1997) and neuroblastoma (Cellai *et al*., 2006, 2010). However, it has been suggested the anti-tumor effect induced by such PPARγ ligands occurs via a PPARγ-independent pathway without the presence of the PPARγ receptors (Abe *et al*., 2002; Lecomte *et al*., 2008). Alternatively, several lines of evidence suggest that activated PPARγ is not a tumor suppressor, but rather functions as an oncogene. First, expression of PPARγ is higher in human prostate cancer cells than in normal prostate tissues (Han and Roman, 2007). Second, PPARγ exhibits a pro-tumor effect in mice bearing a mutation in the *APC* tumor suppressor gene, because PPARγ agonizts increase the frequency and size of colon tumors (Lefebvre *et al*., 1998; Saez *et al*., 1998). Third, PPARγ antagonists have anticancer effects in other cell lines and mouse models (Cui *et al*., 2002; Burton *et al*., 2008).

MicroRNAs have critical roles in many biological processes including cancer by directly interacting with specific mRNAs through base pairing and then inhibiting expression of the target genes through a variety of molecular mechanisms (Bartel, 2009; Croce, 2009; Ventura and Jacks, 2009). The miR-27 family (miR-27a and miR-27b) directly targets PPARγ, and it inhibits adipocyte differentiation (Karbiener *et al*., 2009; Kim *et al*., 2010) and is induced upon inflammation in macrophages (Jennewein *et al*., 2010). Here, we show that miR-27b also targets *PPARγ* in neuroblastoma cells. miR-27b overexpression or *PPARγ* inhibition blocks neuroblastoma growth *in vitro* and *in vivo*. This growth inhibition is associated with decreased expression of *NHE1*, a PPARγ target gene, and a reduced inflammatory response. In contrast, PPARγ inhibits *NHE1* expression, the inflammatory response, and growth of a breast cancer cell line. These results suggest

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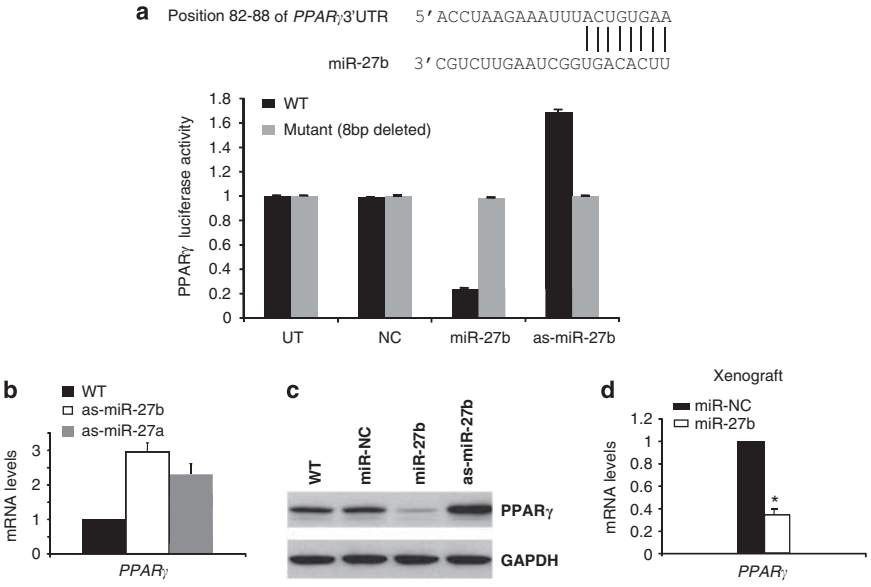

Figure 1 miR-27b targets the 3′ untranslated region (3′UTR) of *PPARγ*. (**a**) Sequence complementarity (vertical lines showing the seed sequence between positions 82–88) between miR-27b and the *PPARγ*. Luciferase activity of reporters containing the wild-type or 8-bp deleted 3′UTR of *PPARγ* 24 h after transfection with miR-27b, antisense (as) against miR-27b or miR negative control or non-transfected cells (UT). (**b**) *PPARγ* mRNA levels in SK-N-AS cells transfected with as-miR-27a (gray bar) or as-miR-27b (white bar). (**c**) Western blot showing *PPARγ* protein levels in cells transfected with the indicated RNAs; levels of GAPDH serve as a loading control. (**d**) *PPARγ* mRNA levels in mouse xenografts (SK-N-AS cells) that are or are not injected with miR-27b. Error bars: s.e.m. $*P<0.05$.

that miR-27b functions as a tumor suppressor, that *PPARγ* promotes tumor formation in neuroblastomas and that cell type-specific regulation of *NHE1* by PPARγ underlies the difference between the oncogenic and tumor suppressing functions of PPARγ in different cell types.

Results

miR-27b inhibits PPARγ expression via its 3′ untranslated region in neuroblastoma

As the miR-27 family (miR-27a and miR-27b) directly targets PPARγ in adipocytes and macrophages (Karbiener *et al*., 2009; Jennewein *et al*., 2010; Kim *et al*., 2010), we examined whether PPARγ is a direct target of miR-27b in a cancer context. Luciferase reporter plasmids containing the wild-type 3′ untranslated region sequence of PPARγ or a deletion mutant (lacking the 8-bp seed sequence) were transfected into the SK-N-AS neuroblastoma cancer cell line with miR-27b or an antisense RNA against miR-27b (as-miR-27b). PPARγ luciferase activity of the wild-type reporter is reduced fivefold upon miR-27b overexpression, whereas it is increased by 60% upon miR-27b inhibition (Figure 1a). In contrast, no changes in PPARγ luciferase activity are observed in the mutant reporter plasmid upon overexpression of miR-27b or as-miR-27b. As expected, antisense-mediated inhibition of either miR-27a or miR-27b results in increased levels of PPARγ mRNA (Figure 1b). In addition, PPARγ protein levels are decreased upon overexpression of miR-27b and increased upon addition of antisense against miR-27b (Figure 1c). Lastly, in 10-day-old tumors generated by injection of SK-N-AS cells in a nude mice, PPARγ mRNA expression is reduced threefold in tumors injected intratumoral with miR-27b, but not with the control miRNA (Figure 1d). Thus, miR-27b inhibits PPARγ expression in neuroblastomas cells.

miR-27b inhibits neuroblastoma cell growth in vitro *and tumor growth in mouse xenografts*

We investigated the role of miR-27b in neuroblastoma cell growth by overexpressing either miR-27b or its antisense RNA. Overexpression of miR-27b or miR-27a inhibits cell growth, whereas overexpression of as-miR-27b or as-miR-27a increases cell growth (Figures 2a). More importantly, in mouse xenografts involving the neuroblastoma cell line, administration of four cycles of miR-27b, but not a control miRNA, strongly reduces tumor growth, whereas tumor growth is enhanced by treatment with as-miR-27b (Figure 2b). These observations are indicative of a tumor suppressive role for miR-27b in neuroblastomas, and they are in accord with studies in other types of cancer. Specifically, miR-27b functions as a tumor suppressor gene in breast cancer, and it is highly expressed in human normal breast tissues (Lu *et al*., 2005) but less expressed in breast cancer tissues (Tsuchiya *et al*., 2006). In addition, miR-27b expression is suppressed in anaplastic thyroid cancer (Braun *et al*., 2010).

miR-27b levels are reduced in neuroblastoma tissues

To examine whether the tumor-suppressor effects of miR-27b in neuroblastoma cell lines are relevant to the human disease, we measured miR-27b RNA levels in tissue samples from human patients. In all, nine cases

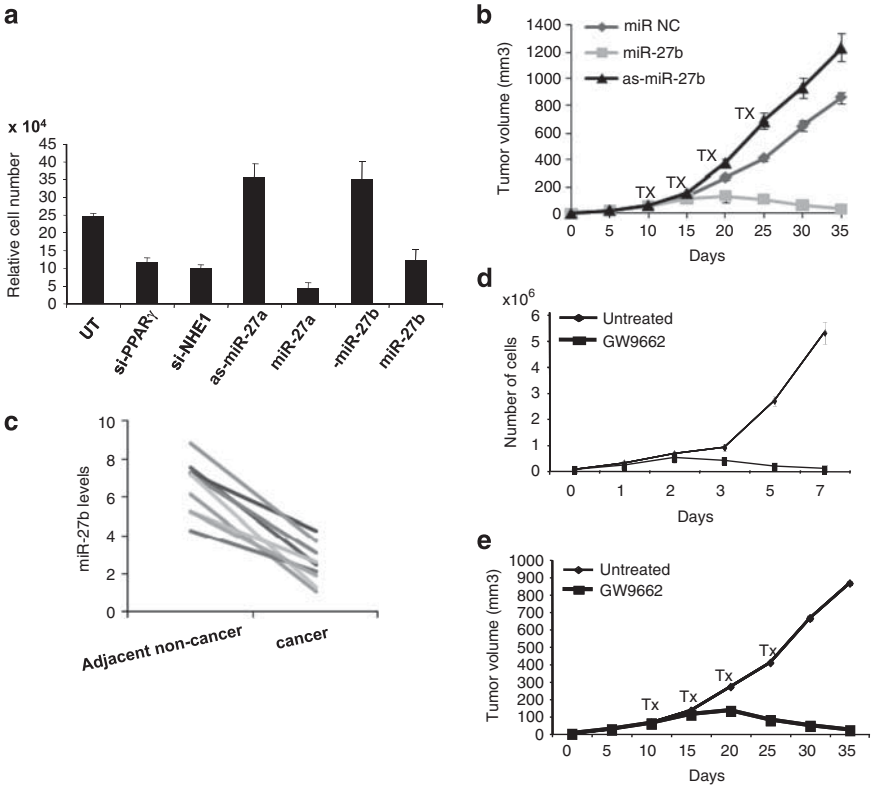

Figure 2 miR-27b through *PPARγ* affecting the cell growth in neuroblastoma cancer *in vitro* and *in vivo*. (**a**) Relative number of viable SK-N-AS cells that were transfected with the indicated RNAs for 24 h and then allowed to grow for an additional 24 h. UT indicates untreated (that is, no siRNA). (**b**) Tumor growth (mean ± s.d.) of mouse xenografts containing neuroblastoma (SK-N-AS) cells after intraperitoneal treatment with miR-27b, as-miR-27b or control miRNA on the indicated number of days after the initial injection of cancer cells. (**c**) miR-27b RNA levels in neuroblastoma and adjacent non-cancer tissues from nine patients, with each line representing an individual patient. (**d**) Growth of SK-N-AS cells in the presence or absence of GW9962 for the indicated number of days. (**e**) Tumor growth (mean ± s.d.) of mouse xenografts containing neuroblastoma (SK-N-AS) cells after intraperitoneal treatment with GW9662 (or no treatment) on the indicated number of days after the initial injection of cancer cells.

tested, miR-27b levels in neuroblastoma tissue were two to threefold lower than in the adjacent non-cancer tissue (Figure 2c). Thus, reduced levels of miR-27b are associated with neuroblastoma.

PPARγ has a tumor-promoting role in neuroblastoma

The functional role of PPARγ activation during cancer development remains controversial, in part because the experiments have been performed with PPARγ agonists or antagonists that may mediate their effects through non-PPARγ mechanisms (see Introduction). To avoid this problem, we inhibited expression of the *PPARγ* gene by an siRNA and found that this resulted in reduced cell viability (Figure 2a). In accord with these experiments, treatment of these neuroblastoma cells with the PPARγ antagonist GW9662 inhibits cell growth *in vitro* (Figure 2d) and in mouse xenografts (Figure 2e). In addition, GW9662 inhibits growth of a different neuroblastoma cell line (SK-N-SH; Supplementary Figure 1). Lastly, as mentioned above, miR-27b acts as a tumor suppressor, providing an independent line of evidence that reduction of PPARγ levels is associated with reduced cancer cell growth. Collectively these observations strongly suggest that PPARγ has a growth-stimulating and tumor-promoting role in neuroblastoma cells.

PPARγ activates NHE1 in neuroblastoma cells

Activation of the pH regulator *NHE1* causes tumors to become more acidic extracellularly and more alkaline intracellularly even during the early stages of neoplastic progression, and hence *NHE1* activation is tumor promoting (Hagag *et al*., 1987; Ober and Pardee, 1987; Siczkowski *et al*., 1994; Reshkin *et al*., 2000). Indeed, si-RNA-mediated inhibition of *NHE1* expression results in reduced growth of SK-N-AS neuroblastoma cells (Figure 2a). *NHE1* expression is directly regulated by binding of PPARγ to target sites in the *NHE1* promoter, and activated PPARγ inhibits *NHE1* expression in breast cancer cell lines (Kumar *et al*., 2009; Venkatachalam *et al*., 2009). These observations are consistent with a number of studies concluding that PPARγ has anti-tumor effects in breast cancer (Mueller *et al*., 1998; Mehta *et al*., 2000; Girnun *et al*., 2002; Kim *et al*., 2006; Kumar *et al*., 2009).

We independently confirmed the anti-tumor effects of PPARγ in breast cancer cells using an isogenic model of cellular transformation involving non-transformed mammary epithelial cells (MCF-10A)(Soule *et al*., 1990) containing ER-Src, a derivative of the Src kinase oncoprotein (v-Src) that is fused to the ligand-binding domain of the estrogen receptor (Aziz *et al*., 1999). Treatment of such cells with tamoxifen rapidly induces

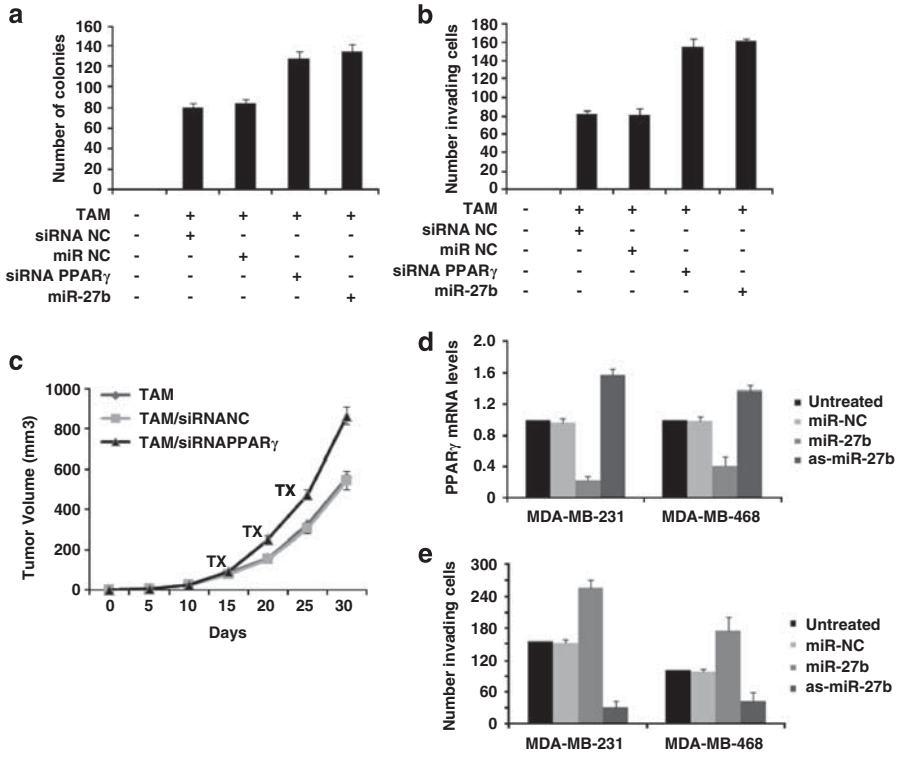

Figure 3 *PPARγ* functions as a tumor suppressor in a isogenic model of transformation in breast cells. (**a**) Colony formation in soft agar of the ER-Src cells that were or were not treated with tamoxifen (TAM) and/or transfected with miR-27b, siRNA against *PPARγ* or control siRNA and miRNA. (**b**) Invasive growth (invading cell/field after wounding) of the cells described in panel (**a**). (**c**) Tumor growth (mean ± s.d.) of mouse xenografts containing transformed ER-Src cells after intraperitoneal treatment with siRNA against *PPARγ* or control siRNA on the indicated number of days after the initial injection of cancer cells. (**d**) *PPARγ* RNA levels in the indicated breast cancer cell lines treated with miR-27b, as-miR-27b or control miRNA. (**e**) Invasive growth in the indicated breast cancer cell lines treated with miR-27b, as-miR-27b or control miRNA.

Src, and morphological transformation is observed within 24–36 h (Hirsch *et al*., 2009; Iliopoulos *et al*., 2009), thereby making it possible to kinetically follow the transition between non-transformed and transformed cells. In this isogenic model, siRNA-mediated inhibition of PPARγ or exogenous expression of miR-27b results in increased tumorigenicity (colonies growing in soft agar; Figure 3a) and invasive growth (MATRIGEL assay; Figure 3b). Furthermore, tumors derived from these transformed ER-Src cells in mouse xenografts grow more quickly upon injection of siRNA against PPARγ (Figure 3c). Similar effects of miR-27b on reducing *PPARγ* expression (Figure 3d) and increasing invasive growth (Figure 3e) are observed in two other breast cancer cells lines (MDA-MB-231 and MDA-MB-468).

In contrast to the results in breast cancer cells, several lines of evidence indicate that PPARγ activates *NHE1* expression in neuroblastoma cells. First, expression of as-miR-27b causes increased *NHE1* expression (Figure 4a) along with increased *PPARγ* expression (Figures 1b and c) in cell culture. Conversely, expression of miR-27b in mouse xenografts reduces *NHE1* (Figure 4b) and *PPARγ* expression (Figure 1d). Second, treatment of neuroblastoma cells with siRNA against PPARγ causes a fourfold decrease in *NHE1* expression levels (Figure 4c). Third, the PPARγ antagonist GW9662 inhibits both *PPARγ* and *NHE1* expression in cell culture (Figure 4d) and in mouse xenografts (Figure 4e). Taken together, these observations suggest that *PPARγ* can activate or inhibit *NHE1* expression in a cell type-specific manner, and that the differential regulation of *NHE1* expression accounts for the opposing tumor-promoting or tumor-inhibiting effects in these different cell types.

miR-27b and PPARγ regulate the inflammatory response in neuroblastoma cells

The inflammatory transcription factor nuclear factor-κB (NF-κB) physically interacts with PPARγ (Chung *et al*., 2000), and there is a great deal of evidence linking NF-κB and inflammation to cancer (Balkwill and Mantovani, 2001; Karin, 2006; Naugler and Karin, 2008; Iliopoulos *et al*., 2009). We therefore examined the effect of miR-27 and PPARγ on the inflammatory response. Inhibition of miR-27b in SK-N-AS neuroblastoma cells increases mRNA levels of four inflammatory factors (interleukin (IL)-1A, Janus kinase 2, IL-6 and IL-1B), whereas expression of miR-27b results in decreased expression (Figure 5a). In addition, mRNA levels of these inflammatory factors are strongly reduced upon siRNA-mediated (Figure 5a) or pharmacological inhibition (GW9662) of PPARγ (Figure 5b). Importantly, the increased expression of inflammatory factors upon

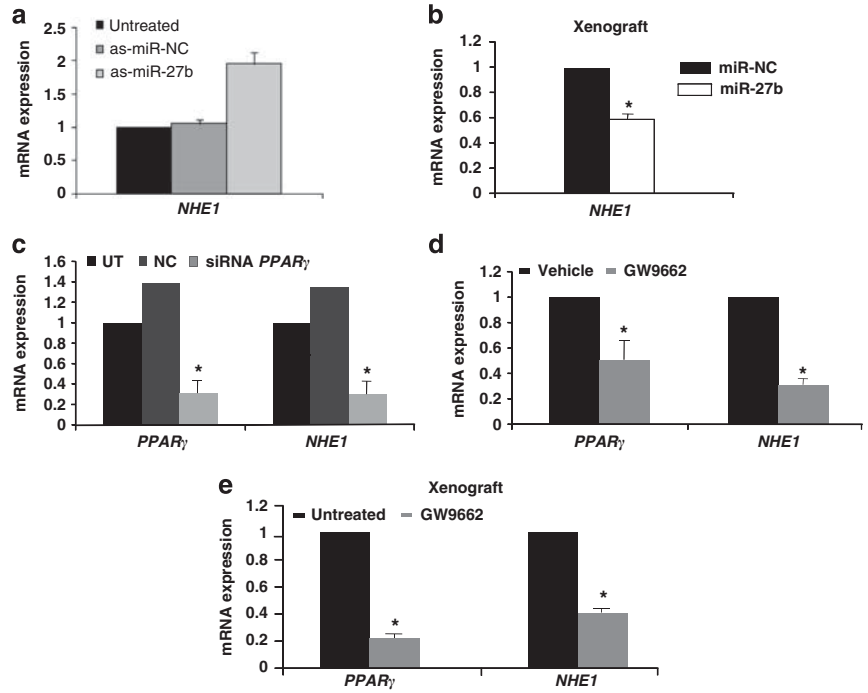

Figure 4 PPARγ promotes cell growth *in vitro* and *in vivo*. (**a**) *NHE1* RNA levels in SK-N-AS cells that were or were not treated with as-miR-27b RNA or control miRNA. (**b**) *NHE1* RNA levels in mouse xenografts (SK-N-AS cells) that are or are not injected with miR-27b. (**c**) *PPARγ* and *NHE1* RNA levels in SK-N-AS cells treated with siRNA against *PPARγ* or control siRNA. (**d**) *PPARγ* and *NHE1* RNA levels in SK-N-AS cells that were or were not treated with GW9662. (**e**) *PPARγ* and *NHE1* RNA levels in mouse xenografts containing SK-N-AS cells that were or were not treated with GW9662. Error bars: s.e.m. $*P<0.05$.

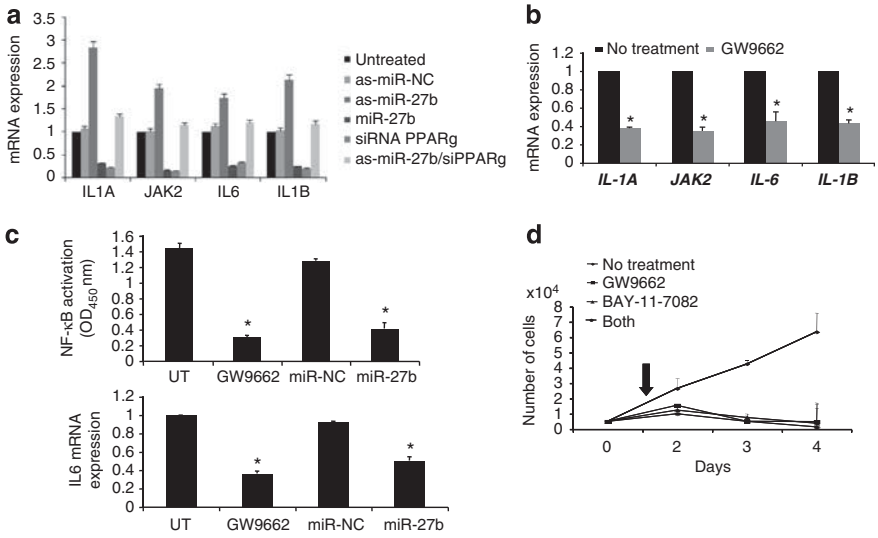

Figure 5 miR-27b through *PPARγ* regulates the NF-κB pathway in neuroblastoma cells and tumors. (**a**) RNA levels of the indicated inflammatory genes in SK-N-AS cells treated with the indicated RNAs. (**b**) RNA levels of the indicated inflammatory genes in SK-N-AS cells that were or were not treated with GW9662. (**c**) NF-κB activity or IL6 RNA levels in tumors from mouse xenografts (SK-N-AS cells) that are treated with miR-27b or control miRNA or GW9662. (**d**) Number of SK-N-AS cells after treatment with the indicated inhibitors. Error bars: s.e.m. $*P<0.05$.

reduction of miR-27b is blocked by simultaneous inhibition of PPARγ (Figure 5a), suggesting that the effects of miR-27b are mediated through PPARγ. In accord with these observations, tumors harvested from the mice either treated with GW9662 or with miR-27b show significantly lower NF-κB activity and reduced IL-6 mRNA expression relative to control groups (Figure 5c). Lastly, neuroblastoma cell growth is inhibited upon treatment with an NF-κB inhibitor (BAY-117082; Figure 5d) at concentrations that do

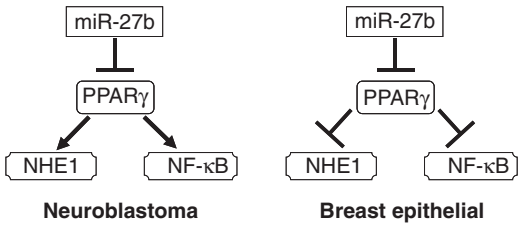

Figure 6 Model. In neuroblastoma, miR-27b inhibits *PPARγ*, which functions as an oncogene that activates downstream targets *NHE1* and NF-κβ in tumor development. In breast cancer cells, *PPARγ* functions as a tumor suppressor that inhibits *NHE1* expression.

not affect the growth of non-transformed cells (Supplementary Figure 2). Thus, miR-27b and PPARγ regulate the inflammatory response in neuroblastoma cells.

Discussion

Our study identifies a molecular pathway important for growth and tumor progression of neuroblastoma cells (Figure 6). Specifically, miR-27b functions as a tumor suppressor by directly inhibiting the expression of PPARγ. Inhibition of PPARγ by miR-27b, si-RNA or a pharmacological antagonist reduces expression of *NHE1* (presumably by direct binding to the promoter region) and the inflammatory response (by an unknown mechanism). Furthermore, inhibition of PPARγ results in reduced cell growth *in vitro* and tumor growth in mouse xenografts, indicating that PPARγ functions as a tumor-promoting factor in neuroblastomas. In accord with this tumor-promoting function, PPARγ stimulates *NHE1* expression and inflammation, both of which are linked to tumor progression in multiple cell types. Our results do not exclude additional cancer-related functions for miR-27b or for PPARγ in neuroblastoma, and indeed these are likely.

Our study also provides new insights on how a transcription factor can act either as an oncogene or as a tumor suppressor depending on the cell type. PPARγ activates *NHE1* expression in neuroblastomas, but it inhibits *NHE1* expression in breast cancer cells, and this discordant regulation of *NHE1*, an oncogenic factor, is linked to tumor suppression in breast cells and tumor promotion in neuroblastomas (Figure 6). There are many examples in which a DNA-binding transcription factor can directly activate or repress genes in a given cell type, or directly activate or repress a given gene in different cell types. We therefore suggest that PPARγ has oncogenic or tumor suppressor functions in different cell types by virtue of cell type-specific regulation of *NHE1* and perhaps other target genes.

Materials and methods

Cell lines
The neuroblastoma cell line SK-N-AS (American Type Culture Collection, Manassas, VA, USA) was maintained in Dulbecco's modified Eagle's medium media (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA), and penicillin/streptomycin (Invitrogen) at 37 °C with 5% CO_2. The breast epithelial cell line MCF-10A cells containing the ER-Src fusion protein was grown in Dulbecco's modified Eagle's medium/F12 medium supplemented with 5% donor horse serum, 20 ng/ml epidermal growth factor, 10 mg/ml insulin, 100 mg/ml hydrocortisone, 1 ng/ml cholera toxin and 50 units/ml penicillin/streptomycin, with the addition of puromycin (Hirsch *et al*., 2009; Iliopoulos *et al*., 2009). To induce transformation, the Src oncogene was activated by the addition of 1 mM tamoxifen (Sigma, St Louis, MO, USA correct) for 36 h.

Luciferase assays
The firefly luciferase reporter plasmids contained the entire wild-type 3′ untranslated region of *PPARγ* (Genecopeia Inc., Rockville, MD, USA) or a mutated derivative deleted for the 8 bp seed sequence deleted generated by inverse-PCR (Supplementary Table 1). The *Renilla* plasmids (0.8 μg) were co-transfected into SK-N-AS cells either with 33 nM of as-miR-27b (AM10750, Ambion, Austin, TX, USA), miR-27b (C-300589-05, Dharmacon, Lafayette, CO, USA) or with non-targeting control (NC; PM11440, Ambion) using Lipofectamine 2000 (Invitrogen) to the cells. The PPARγ luciferase activity of the luciferase vector construct only (UT) was normalized to one and the other transfection combinations were compared with UT. Cells were harvested 48 h after transfection and assayed using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).

RNA analysis
RNA was purified by the Trizol method (Invitrogen), treated with RNase-free DNase (Ambion) and reverse transcribed with using SuperScript III RT (Invitrogen) to generate cDNA. RNA levels were determined by SYBR Green-based real-time–PCR of the cDNA, with the level of β-actin used as a loading control. Each sample was run in triplicate, and the data represent the mean ± s.d. of three independent experiments. PCR primers used for these analyses are shown in Supplementary Table 1.

Western blotting
The total protein (50 μg) from neuroblastoma cells was isolated by standard methods in radioimmuno precipitation assay buffer (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium, deoxycholate and 0.1% sodium dodecyl sulfate), electrophoretically separated and transferred to nitrocellulose filters. The filters were incubated overnight at 4 °C with anti-PPARγ (1:200; ab27649, Abcam Inc., Cambridge, UK) and anti-α-tubulin (1:3000; Clone DM1A, Sigma). The density of the bands was quantified and normalized by the loading control, γ-tubulin.

Genetic and pharmacological analysis of cell growth
For genetic analysis, SK-N-AS cells seeded in 6- or 12-well plates were transfected with 100 nM miRNAs, antisense (as)-miRNAs or siRNAs using the siPORT NeoFX transfection agent (Ambion) and incubated for 24 h. The number of viable cells was measured at various times after this initial incubation period. For pharmacological analysis, cells were seeded in 24-well plates for an initial 20-h incubation period, after which time they were treated with medium containing 15 μM GW9662 (PPARγ antagonist; Cayman Chemical, Ann Arbor, MI, USA), a 5 μM BAY-11-72 (NF-kB inhibitor; Sigma) or

dimethyl sulfoxide (vehicle). Medium containing these inhibitors was changed every 24 h.

Soft agar colony and invasion assays

The soft agar colony and MATRIGEL invasion assays for MCF-10A-ER-Src cells were performed as described previously (Iliopoulos *et al*., 2009; Hirsch *et al*., 2010).

Xenograft experiments

SK-N-AS cells (5×10^6) were injected into the right flank of nu/nu mice (Charles River Laboratories, Wilmington, MA, USA), all of which developed tumors in 10 days with size of $\sim 60\,mm^3$. The mice were randomly distributed into groups (typically four mice per group) and treated with miR-27b (100 nM), miRNA negative control (miR-NC; 100 nM), GW9662 (2.5 mg/kg) or dimethyl sulfoxide (0.1 ml/10 g body weight). All treatments were administered intraperitoneally injection every 5 days starting on day 10–25 for four cycles. Tumor volumes were monitored every 5 days. Tumors were harvested on day 35 for mRNA analysis of *PPARγ* and *NHE1* and for measurements of NF-kB activity (ActivELISA kit IMK-503, Imgenex, San Diego, CA, USA). All mouse experiments were performed according to the Institutional Animal Care and Use Committee procedures and guidelines of the Tufts University.

Conflict of interest

The authors declare no conflict of interest.

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References

Abe A, Kiriyama Y, Hirano M, Miura T, Kamiya H, Harashima H *et al*. (2002). Troglitazone suppresses cell growth of KU812 cells independently of PPARgamma. *Eur J Pharmacol* **436**: 7–13.

Aziz N, Cherwinski H, McMahon M. (1999). Complementation of defective colony-stimulating factor 1 receptor signaling and mitogenesis by Raf and v-Src. *Mol Cell Biol* **19**: 1101–1115.

Balkwill F, Mantovani A. (2001). Inflammation and cancer: back to Virchow? *Lancet* **357**: 539–545.

Bartel DP. (2009). MicroRNAs: target recognition and regulatory functions. *Cell* **136**: 215–233.

Braun J, Hoang-Vu C, Dralle H, Huttelmaier S. (2010). Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. *Oncogene* **29**: 4237–4244.

Burton JD, Goldenberg DM, Blumenthal RD. (2008). Potential of peroxisome proliferator-activated receptor gamma antagonist compounds as therapeutic agents for a wide range of cancer types. *PPAR Res* **2008**: 494161.

Cellai I, Benvenuti S, Luciani P, Galli A, Ceni E, Simi L *et al*. (2006). Antineoplastic effects of rosiglitazone and PPARgamma transactivation in neuroblastoma cells. *Br J Cancer* **95**: 879–888.

Cellai I, Petrangolini G, Tortoreto M, Pratesi G, Luciani P, Deledda C *et al*. (2010). *In vivo* effects of rosiglitazone in a human neuroblastoma xenograft. *Br J Cancer* **102**: 685–692.

Chattopadhyay N, Singh DP, Heese O, Godbole MM, Sinohara T, Black PM *et al*. (2000). Expression of peroxisome proliferator-activated receptors (PPARS) in human astrocytic cells: PPARgamma agonists as inducers of apoptosis. *J Neurosci Res* **61**: 67–74.

Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G *et al*. (2000). Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B. *J Biol Chem* **275**: 32681–32687.

Croce CM. (2009). Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* **10**: 704–714.

Cui Y, Miyoshi K, Claudio E, Siebenlist UK, Gonzalez FJ, Flaws J *et al*. (2002). Loss of the peroxisome proliferation-activated receptor gamma (PPARgamma) does not affect mammary development and propensity for tumor formation but leads to reduced fertility. *J Biol Chem* **277**: 17830–17835.

Girnun GD, Smith WM, Drori S, Sarraf P, Mueller E, Eng C *et al*. (2002). APC-dependent suppression of colon carcinogenesis by PPARgamma. *Proc Natl Acad Sci USA* **99**: 13771–13776.

Hagag N, Lacal JC, Graber M, Aaronson S, Viola MV. (1987). Microinjection of ras p21 induces a rapid rise in intracellular pH. *Mol Cell Biol* **7**: 1984–1988.

Han S, Roman J. (2007). Peroxisome proliferator-activated receptor gamma: a novel target for cancer therapeutics? *Anticancer Drugs* **18**: 237–244.

Han SW, Greene ME, Pitts J, Wada RK, Sidell N. (2001). Novel expression and function of peroxisome proliferator-activated receptor gamma (PPARgamma) in human neuroblastoma cells. *Clin Cancer Res* **7**: 98–104.

Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M *et al*. (2010). A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. *Cancer Cell* **17**: 348–361.

Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. (2009). Metformin selectively targets cancer stem cells and acts together with chemotherapy to blocks tumor growth and prolong remission. *Cancer Res* **69**: 7507–7511.

Iliopoulos D, Hirsch HA, Struhl K. (2009). An epigenetic switch involving NF-kB, lin 28, let-7 microRNA, and IL6 links inflammation to cell transformation. *Cell* **139**: 693–706.

Jennewein C, von Knethen A, Schmid T, Brune B. (2010). MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor gamma (PPARgamma) mRNA destabilization. *J Biol Chem* **285**: 11846–11853.

Karbiener M, Fischer C, Nowitsch S, Opriessnig P, Papak C, Ailhaud G *et al*. (2009). microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. *Biochem Biophys Res Commun* **390**: 247–251.

Karin M. (2006). Nuclear factor-kappaB in cancer development and progression. *Nature* **441**: 431–436.

Kim KY, Kim SS, Cheon HG. (2006). Differential anti-proliferative actions of peroxisome proliferator-activated receptor-gamma agonists in MCF-7 breast cancer cells. *Biochem Pharmacol* **72**: 530–540.

Kim SY, Kim AY, Lee HW, Son YH, Lee GY, Lee JW *et al*. (2010). miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARgamma expression. *Biochem Biophys Res Commun* **392**: 323–328.

Kumar AP, Quake AL, Chang MK, Zhou T, Lim KS, Singh R *et al*. (2009). Repression of NHE1 expression by PPARgamma activation is a potential new approach for specific inhibition of the growth of tumor cells *in vitro* and *in vivo*. *Cancer Res* **69**: 8636–8644.

Lecomte J, Flament S, Salamone S, Boisbrun M, Mazerbourg S, Chapleur Y *et al*. (2008). Disruption of ERalpha signalling pathway

by PPARgamma agonists: evidences of PPARgamma-independent events in two hormone-dependent breast cancer cell lines. *Breast Cancer Res Treat* **112**: 437–451.

Lefebvre AM, Chen I, Desreumaux P, Najib J, Fruchart JC, Geboes K *et al*. (1998). Activation of the peroxisome proliferator-activated receptor gamma promotes the development of colon tumors in C57BL/6J-APCMin/+ mice. *Nat Med* **4**: 1053–1057.

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D *et al*. (2005). MicroRNA expression profiles classify human cancers. *Nature* **435**: 834–838.

Mehta RG, Williamson E, Patel MK, Koeffler HP. (2000). A ligand of peroxisome proliferator-activated receptor gamma, retinoids, and prevention of preneoplastic mammary lesions. *J Natl Cancer Inst* **92**: 418–423.

Michalik L, Desvergne B, Wahli W. (2004). Peroxisome-proliferator-activated receptors and cancers: complex stories. *Nat Rev Cancer* **4**: 61–70.

Morosetti R, Servidei T, Mirabella M, Rutella S, Mangiola A, Maira G *et al*. (2004). The PPARgamma ligands PGJ2 and rosiglitazone show a differential ability to inhibit proliferation and to induce apoptosis and differentiation of human glioblastoma cell lines. *Int J Oncol* **25**: 493–502.

Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M *et al*. (1998). Terminal differentiation of human breast cancer through PPAR gamma. *Mol Cell* **1**: 465–470.

Naugler WE, Karin M. (2008). NF-kappaB and cancer-identifying targets and mechanisms. *Curr Opin Genet Dev* **18**: 19–26.

Nwankwo JO, Robbins ME. (2001). Peroxisome proliferator-activated receptor- gamma expression in human malignant and normal brain, breast and prostate-derived cells. *Prostaglandins Leukot Essent Fatty Acids* **64**: 241–245.

Ober SS, Pardee AB. (1987). Intracellular pH is increased after transformation of Chinese hamster embryo fibroblasts. *Proc Natl Acad Sci USA* **84**: 2766–2770.

Reshkin SJ, Bellizzi A, Caldeira S, Albarani V, Malanchi I, Poignee M *et al*. (2000). Na+/H+ exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. *FASEB J* **14**: 2185–2197.

Saez E, Tontonoz P, Nelson MC, Alvarez JG, Ming UT, Baird SM *et al*. (1998). Activators of the nuclear receptor PPARgamma enhance colon polyp formation. *Nat Med* **4**: 1058–1061.

Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB *et al*. (1998). Differentiation and reversal of malignant changes in colon cancer through PPARgamma. *Nat Med* **4**: 1046–1052.

Siczkowski M, Davies JE, Ng LL. (1994). Activity and density of the Na+/H+ antiporter in normal and transformed human lymphocytes and fibroblasts. *Am J Physiol* **267**: C745–C752.

Soule HD, Maloney TM, Wolman SR, Peterson WD, Brenz R, McGrath CM *et al*. (1990). Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF10. *Cancer Res* **50**: 6075–6086.

Tontonoz P, Singer S, Forman BM, Sarraf P, Fletcher JA, Fletcher CD *et al*. (1997). Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. *Proc Natl Acad Sci USA* **94**: 237–241.

Tsuchiya Y, Nakajima M, Takagi S, Taniya T, Yokoi T. (2006). MicroRNA regulates the expression of human cytochrome P450 1B1. *Cancer Res* **66**: 9090–9098.

Venkatachalam G, Kumar AP, Yue LS, Pervaiz S, Clement MV, Sakharkar MK. (2009). Computational identification and experimental validation of PPRE motifs in NHE1 and MnSOD genes of human. *BMC Genomics* **10**(Suppl 3): S5.

Ventura A, Jacks T. (2009). MicroRNAs and cancer: short RNAs go a long way. *Cell* **136**: 586–591.

Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)