Opposing control of rhabdomyosarcoma growth and differentiation by myogenin and interleukin 4

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Abstract

Rhabdomyosarcoma is a tumor of striated muscle origin that displays defective myogenic differentiation. Terminal myogenesis switches off cell proliferation and migration, hence, the promotion of rhabdomyosarcoma differentiation should antagonize tumor growth and metastasis. Terminal myogenesis is controlled by cell-intrinsic myogenic transcription factors like myogenin and environmental mediators like interleukin 4 (IL-4). We studied whether the expression of myogenin or exposure to IL-4 could promote the myogenesis of poorly differentiating human rhabdomyosarcoma cells RD/12. Forced expression of myogenin amplified myosin expression and the formation of myotube-like elements, inhibited cell migration, and reduced the growth of local tumors and liver metastases in immunodepressed mice. In contrast, exposure to IL-4 promoted cell proliferation and survival, especially at high cell density, inhibited myogenin expression, and myogenesis. Moreover, IL-4 stimulated the directed migration of cells with low myogenin levels, but not of cells with higher (spontaneous or forced) levels. Thus, IL-4, which was known to promote late stages of normal myogenesis, favors growth and migration, and inhibits further differentiation of the myogenic stages attained by rhabdomyosarcoma cells. Strategies to increase myogenin expression and block IL-4 could simultaneously reduce growth and migration, and enhance terminal differentiation of rhabdomyosarcoma, thus contributing to the control of tumor growth and metastatic spread. [Mol Cancer Ther 2009;8(4):754–61]

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Introduction

Rhabdomyosarcomas are tumors of the myogenic lineage containing a variable proportion of cells at different stages of differentiation, ranging from small, proliferating myoblast-like cells (rhabdomyoblasts) to large multinuclear elements resembling myotubes (rhabdomyotubes; ref. 1). Rhabdomyoblasts express myogenic factors like MyoD and myogenin and intermediate filaments vimentin and myosin, rhabdomyotubes express contractile proteins such as myosin isoforms (2–4). This phenotype can be observed in human and experimental tumors, both *in vivo* and *in vitro*.

Normal cell differentiation along the myogenic pathway leads to the formation of postmitotic myotubes. Neoplastic differentiation mostly follows the same pathway, but with a much less efficient formation of rhabdomyotubes and the invariable persistence of proliferating rhabdomyoblasts. In general, more differentiated elements in a rhabdomyosarcoma cell population do not proliferate (or proliferate poorly), nor initiate tumor formation *in vivo*. Hence, the overall proliferative and tumorigenic capacity of rhabdomyosarcomas is inversely related to the degree of myogenic differentiation (4, 5).

The analysis of cloned rhabdomyosarcoma cell populations revealed a considerable differentiative heterogeneity, from undifferentiated cells expressing only the most basic mesenchymal markers to highly differentiated clones which could not be propagated *in vitro* because they invariably and homogeneously underwent terminal differentiation (6). It can be said that the coexistence of tumor-initiating cancer stem cells and non–tumor-initiating cells is a concept that permeated the study of rhabdomyosarcoma differentiation well before it became popular for other tumors like mammary or colorectal carcinoma.

The therapeutic implication of the impaired differentiation of rhabdomyosarcoma is that a restoration, or at least an enhancement, of myogenic differentiation is expected to inhibit tumorigenicity and malignancy. The unrestricted proliferation of rhabdomyosarcoma cells is supported by a redundancy of autocrine circuits, including basic fibroblast growth factor, insulin-like growth factors, epidermal growth

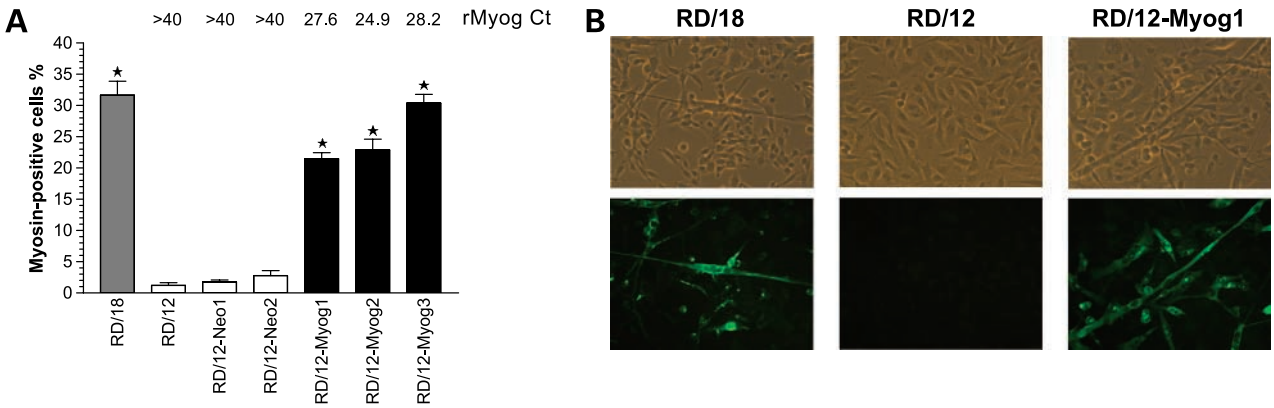

Figure 1. Forced expression of myogenin induces myogenic differentiation of RD/12 rhabdomyosarcoma cells. **A,** quantitation of cellular elements expressing embryonic myosin after 11 d of culture in DMEM + 2% horse serum. *Columns,* mean of four to five independent determinations; *bars,* SE. *Top,* expression of gene-transduced myogenin as determined by real-time reverse transcription PCR, expressed as threshold cycles (*Ct*), a measure inversely proportional to expression level. *Stars,* values significantly different from RD/12 cells ($P < 0.01$ at least by Student's *t* test); **B,** appearance of rhabdomyotubes (*top*) and expression of embryonic myosin (*bottom*) in nontransduced and myogenin-transduced RD/12 cells in comparison with RD/18 cells.

factor, hepatocyte growth factor, connective tissue growth factor, and vascular endothelial growth factor (7–12). This led us and others to explore the induction of rhabdomyosarcoma differentiation through a blockade of autocrine circuits by means of monoclonal antibodies, antisense oligonucleotides, and small interfering RNAs (8, 9, 11, 13). In most instances, the treatments blocked cell proliferation but did not enhance differentiation, indicating that the latter may control the former, but not vice versa.

More recently, our attention was drawn to two classes of molecules whose connection with rhabdomyosarcoma has not been explored in depth, in one case because they were not known to act on myogenesis, in the other because they were taken for granted in this tumor. Interleukins (IL) are normally associated with the immune systems and leukocytes, however, myogenic cells can both release and respond to various cytokines (14–16), and IL-4 was recently shown to induce terminal differentiation of normal muscle cells (17). On the other side, myogenic transcription factors are thought to be uniformly expressed by rhabdomyosarcoma cells, in fact, MyoD expression was proposed early on as a universal marker of the embryonal rhabdomyosarcoma histotype (18, 19). However, this neglect disregards the fact that myogenic transcription factors are the master controllers of myogenesis, and that rhabdomyosarcomas contain cell populations displaying huge quantitative differences in their expression (19–22). In this work, we studied whether forced expression of myogenin or exposure to recombinant IL-4 can indeed modify myogenic differentiation of human rhabdomyosarcoma cells and their ability to initiate tumors and metastases.

Materials and Methods

Cells

RD/12 and RD/18 clonal cell lines were derived in our laboratory by random cloning from the human embryonal rhabdomyosarcoma cell line RD (5) and were used between the 16th and the 29th *in vitro* passage. CCA cell line and RC2 clone (11) were obtained in our laboratory from one embryonal and one alveolar rhabdomyosarcoma, respectively. Media constituents and culture conditions were reported previously (11).

IL-4 Treatment

Recombinant human IL-4 (IL-4) was purchased from PeproTech EC. To evaluate IL-4 effects, cells were cultured in a low serum content medium, DMEM supplemented with 2% horse serum. Cells were seeded in replicates in six-well Multiwell plates (Falcon Plastics) at 8×10^3 cells/cm^2 in standard culture medium and incubated overnight to allow cell attachment, then medium was replaced with DMEM + 2% horse serum in the absence or presence of 10 ng/mL of IL-4. Cells were cultured up to 15 days under the same experimental conditions, with medium renewal every 3 to 4 days. At various time intervals, replicated cultures with and without IL-4 were used to evaluate cell growth, proliferation, differentiation, and apoptosis.

Cell Growth, Proliferation, Apoptosis, and Differentiation

For the evaluation of cell growth, cells were harvested by trypsin-EDTA treatment and cell number and viability determined through erythrosin dye exclusion and hemocytometer count. Cell proliferation was studied by bromodeoxyuridine (Sigma) incorporation as reported (11). For the evaluation of apoptosis, cells were harvested without discarding cells in the supernatants and apoptosis was evaluated by Hoechst 33342 staining (Merck; ref. 11). To evaluate the level of myogenic differentiation, cells grown in DMEM + 2% horse serum were harvested at various time intervals, counted and cytocentrifuged samples prepared, fixed, and stained by indirect immunofluorescence using the antiembryonic myosin monoclonal antibody BF-G6 (23) as previously reported (6). The percentage of myosin-positive cells was determined in a Leica DM microscope. At least 400 cell elements in random fields were analyzed.

Table 1. Tumor growth of myogenin-transduced rhabdomyosarcoma cells in nude mice

Cells	Tumor growth		
	Incidence	Median latency (d)	Latency range
RD/12	5/10 (50%)	55	55–61
RD/12-Neo2	6/10 (60%)	46	46–53
RD/12-Myog1	0/10* (0%)		
RD/12-Myog3	0/10* (0%)		

NOTE: Nude mice received 10^7 cells s.c., tumor growth was monitored for 12 wk.
*$P < 0.05$ at least vs. RD/12 and RD/12-Neo2 (Fisher's exact test).

Myogenin Gene Transduction

Gene transduction was done with plasmid MD-R1-myogenin, derived from a previously described vector (24) and kindly provided by Dr. Marco Crescenzi, Regina Elena Cancer Institute, Rome, Italy. MD-R1-myogenin carries the 1.4 kb rat myogenin cDNA under the control of the SV40 promoter and a neo^r gene expression cassette. Transfection was done by calcium phosphate precipitation (Invitrogen) with 2 μg of plasmid. Selection for stable transfectants was obtained through culture with DMEM + 20% fetal bovine serum containing 500 μg/mL of G418 (Invitrogen). Single transfectant colonies were isolated using sterile glass cloning cylinders; control clones were isolated from cells transduced with the neomycin resistance gene alone. Myogenin expression was assessed by real-time reverse transcription-PCR (11, 22).

Cell Motility

Migration assay was performed in Transwell chambers (Costar) with 8 μm pore size, polyvinylpirrolidone-free polycarbonate filters. Serum-free DMEM alone or supplemented with 10 ng/mL of recombinant human IL-4 was put in the lower compartment; 5×10^5 cells were seeded in serum-free DMEM in the upper compartment of the Transwell chambers, and incubated for 18 h. A shorter time period (6 h) did not lead to significant migration. Cells which migrated through the filter to reach the lower chamber were counted at the inverted microscope.

Mice

Athymic Crl:CD1-*Foxn1*^{nu} (nude) mice were purchased from Charles River Italy. Rag2$^{-/-}$;$\gamma_c^{-/-}$ breeders were kindly given by Dr. M. Ito of the Central Institute for Experimental Animals, Kawasaki, Japan; mice were then bred in our animal facilities under sterile conditions. All animal experiments were authorized by the Institutional Review Board of the University of Bologna and were done according to Italian and European guidelines.

Tumor and Metastasis Growth

Individually tagged virgin female mice 6 to 9 weeks of age were used for the experiments. Local tumors were induced by the s.c. injection of 10^7 viable cells, tumor growth was assessed weekly. For the induction of lung micrometastases, mice received 2×10^6 cells i.v. and were sacrificed 3 months later, and subjected to an accurate necropsy. Lungs were stained with black India ink to better outline metastases and fixed in Fekete's solution. Lung and liver metastases were counted under a dissection microscope.

IL-4 Receptor Level and Signaling

Rhabdomyosarcoma cells were lysed *in situ* for 30 min on ice with 50 mmol/L of Tris-HCl (pH 7.5), 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol, 150 mmol/L of NaCl, 1 mmol/L of EDTA, plus phosphatase and protease inhibitors (all reagents were purchased from Sigma). For the study of IL-4 receptor activation, cells were treated with 10 ng/mL of recombinant human IL-4 in serum-free DMEM for 45 min before lysis. Total protein concentration was determined by DC Protein Assay (Bio-Rad). Western blot analysis was performed as previously described (11). Mouse anti-human IL-13 Rα1 monoclonal antibody (clone 419718, 2 μg/mL), goat anti-human IL-4 Rα antibody (0.2 μg/mL), rabbit anti–phospho-STAT6 (Y641) antibody (0.5 μg/mL; R&D Systems), rabbit anti-total STAT6 (1.5 μg/mL, M-20; Santa Cruz Biotechnology), and rabbit anti-actin (Sigma) were used as primary antibodies. TBS containing 0.1% Tween 20 and 5% nonfat dry milk was used for the evaluation of actin, IL-4 Rα, and IL-13 Rα expression, whereas Tween 20 was lowered to 0.05% for the study of total STAT6 and phospho-STAT6 expression. Protein presence was detected through incubation with the respective horseradish peroxidase–labeled secondary antibodies (Santa Cruz Biotechnology) followed by a colorimetric reaction (Opti-4CN Substrate kit; Bio-Rad).

Results

Myogenin Controls Rhabdomyosarcoma Differentiation

RD/12 and RD/18 are human rhabdomyosarcoma clones selected for their diverging differentiation potential along the myogenic pathway (5). Under continuous culture conditions, both clones show a poorly differentiated phenotype, defined by the expression of desmin and the almost complete absence of myosin isoforms. Serum starvation triggers myogenic differentiation of RD/18, but not of RD/12 cells. Differentiated cultures contain a sizeable proportion of cells expressing myosin and a small proportion of myotube-like multinuclear elements (Fig. 1B; ref. 5).

Table 2. Metastatic spread of myogenin-transduced rhabdomyosarcoma cells in Rag2$^{-/-}$;$\gamma_c^{-/-}$ mice

Cells	Lung metastasis			Liver metastasis		
	Incidence	Median	Range	Incidence	Median	Range
RD/12	3/5 (60%)	2	0–17	5/5 (100%)	125	74–218
RD/12-Neo2	3/4 (75%)	2	0–2	4/4 (100%)	134	109–229
RD/12-Myog1	4/5 (80%)	2	0–31	5/5 (100%)	30*	23–36
RD/12-Myog3	2/4 (50%)	1	0–1	4/4 (100%)	60*	32–89

NOTE: Mice received 2×10^6 cells i.v. and were sacrificed 10 wk later.
*$P < 0.05$ vs. RD/12 and RD/12-Neo2 (Wilcoxon rank sum test).

Figure 2. Metastatic spread of RD/12 and RD/12-Myog cells to the liver of $Rag2^{-/-};\gamma_c^{-/-}$ mice. Three representative fixed and dissected livers are shown for each cell type. Metastasis counts are shown in Table 2.

Earlier microarray studies identified a possible molecular determinant of the blockage of differentiation of RD/12 cells in the low expression of the myogenic factor myogenin (22). To study whether myogenin indeed played a causal role, we transduced RD/12 cells with a rat myogenin gene to distinguish endogenous from transgenic gene expression, and selected a set of clones showing significant myogenin expression levels, i.e., RD/12-Myog1, RD/12-Myog2, and RD/12-Myog3 (Fig. 1A).

Myogenic differentiation after serum starvation was greatly amplified in RD/12-Myog cells, resulting in the expression of myosin in ~30% of cells (Fig. 1A and B), and in the appearance of multinuclear myotube-like elements (Fig. 1B). Myogenic differentiation of RD/12-Myog clones was similar to that of differentiation-proficient RD/18 cells, and was largely superior to that of deficient RD/12 parent cells and control transfectant clones RD/12-Neo1 and RD/12-Neo2.

Myogenin Expression Inhibits Tumor Growth and Metastatic Spread

The myogenic differentiation pathway culminates with postmitotic and multinuclear cells (25); hence, the differentiation competence of rhabdomyosarcoma cells could determine their neoplastic potential (4). The tumorigenicity of RD/12-Myog cells in athymic nude mice was strongly impaired with respect to RD/12 or RD/12-Neo cells (Table 1). After i.v. injection, both RD/12-Myog and RD/12 produced lung nodules only in a minority of nude mice (~35%). We recently found that double knockout mice lacking Rag2 and the γ common cytokine receptor subunit ($Rag2^{-/-};\gamma_c^{-/-}$ mice) allow a better expression of human sarcoma malignancy thanks to the absence of T, B, and NK cell immunity.[4] We then challenged $Rag2^{-/-};\gamma_c^{-/-}$ mice i.v. with RD/12 and RD/12-Myog cells to better study their metastatic potential. Table 2 shows that this new immunodeficient host confirmed the almost equal lung colonization potential of both cell types, but uncovered a significant inhibition in liver metastasization induced by myogenin expression. Visual inspection of livers (Fig. 2) illustrates the huge reduction in total metastatic burden in mice challenged with RD/12-Myog cells.

Altogether, *in vivo* results indicate that myogenin expression inhibits the tumorigenicity and metastatic ability of human rhabdomyosarcoma. Furthermore, the site specificity of this phenomenon, which was observed in local tumors and liver metastases, but not in lung metastases, hints at the involvement of microenvironmental factors.

IL-4 Stimulates Rhabdomyosarcoma Growth and Inhibits Myogenic Differentiation

It was recently suggested that IL-4, a cytokine mainly produced by type 2 helper T cells, could stimulate the maturation of normal myotubes (17). This differentiation step is deficient in all rhabdomyosarcomas because it entails the transition from proliferation-competent cells to nonproliferating, terminally differentiated multinuclear elements. Therefore, we studied whether IL-4 might enhance the terminal differentiation of human rhabdomyosarcoma cells.

Unexpectedly, IL-4 actually promoted rhabdomyosarcoma cell growth (Fig. 3A) and significantly inhibited myogenin expression (Fig. 3B and D) and myogenic differentiation (Fig. 3C). Stimulation of cell growth was especially evident in semiconfluent and confluent cultures, which in the presence of IL-4, failed to show the usual plateau at later time points (Fig. 3A). We took advantage of the early visibility of this phenomenon in RD/12 to analyze the two determinants of cell number, cell proliferation, and apoptosis. We found that IL-4 simultaneously stimulated cell proliferation and inhibited apoptosis. At 4 days of culture, we observed a 91% increase in RD/12 cell proliferation and a 40% reduction in spontaneous apoptosis (data not shown). In summary, IL-4

[4] Lollini et al., in preparation.

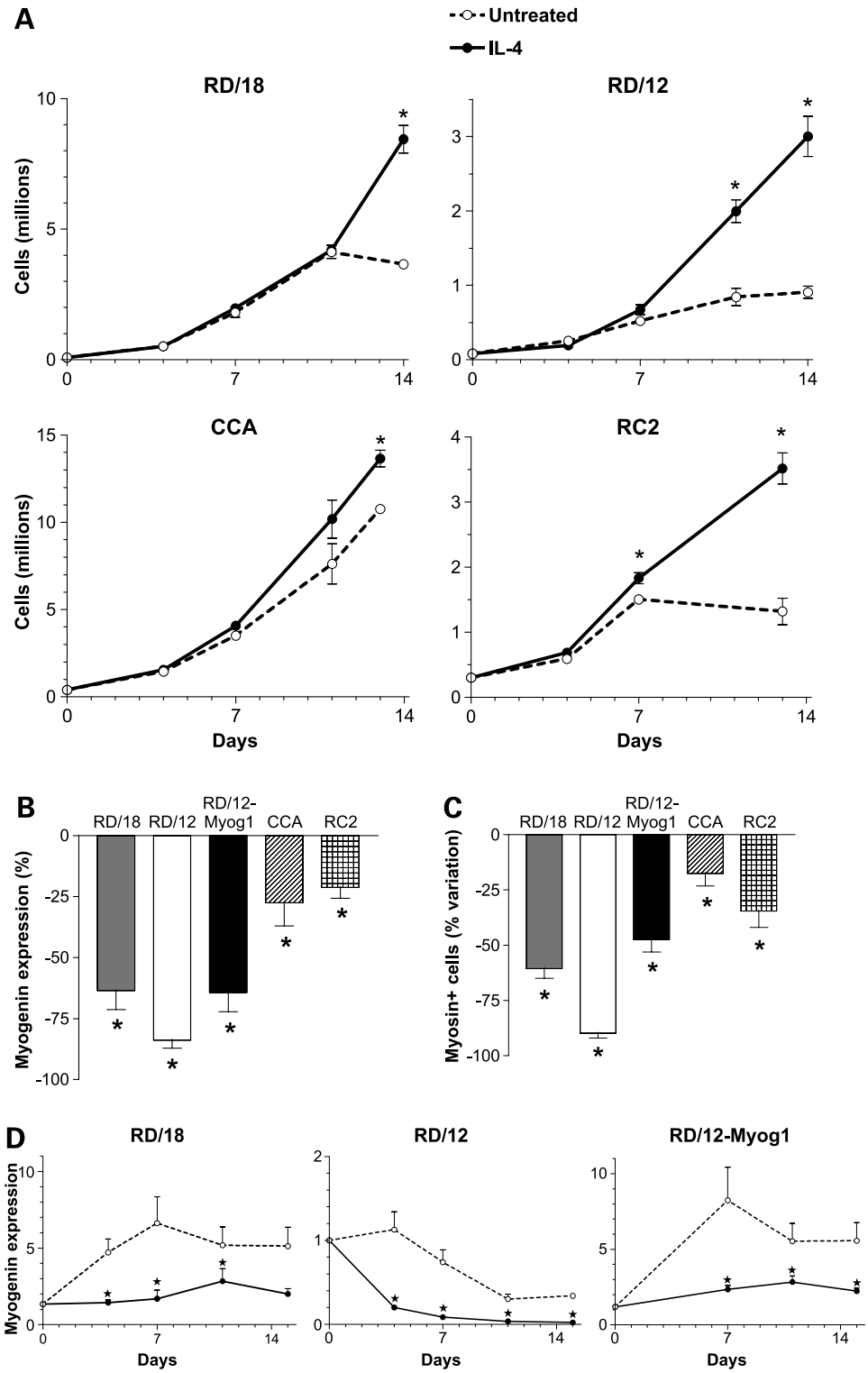

Figure 3. IL-4 stimulates the growth and inhibits myogenic differentiation of rhabdomyosarcoma cells. **A** and **D,** untreated cells (*open symbols + dashed line*), and IL-4–treated (10 ng/mL) cells (*closed symbols + continuous line*). **A,** growth curves of RD/18, RD/12, CCA, and RC2 cells cultured in DMEM + 2% horse serum. **B,** IL-4–induced decrease in the expression of myogenin. *Columns,* percentage of reduction of relative expression as evaluated by quantitative real-time reverse transcription PCR, mean of three to seven independent determinations; *bars,* SE. **C,** IL-4–induced decrease in the proportion of cells expressing embryonic myosin. *Columns,* mean of four to eight independent determinations; *bars,* SE. **D,** kinetics of myogenin expression reported as fold change relative to RD/12 cells at day 0, as determined by real-time reverse transcription PCR. *Stars,* significant differences between IL-4–treated and untreated cultures ($P < 0.05$ at least by the paired Student's t test).

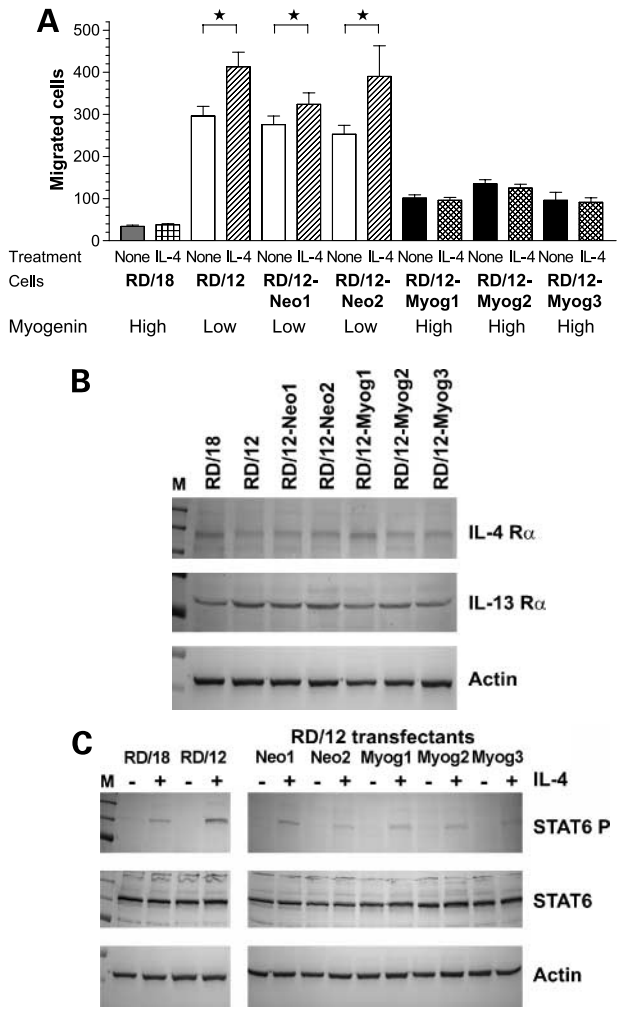

Figure 4. **A**, forced expression of myogenin inhibited RD/12 cell migration and blocked directed migration toward IL-4. *Columns,* mean number of cells migrated in the lower compartment containing medium with or without IL-4, four independent replicates; *bars,* SE. Migration of RD/12-Myog cells was significantly lower than parental RD/12 cells ($P < 0.001$, Student's *t* test); IL-4 significantly (*stars,* $P < 0.05$, Student's *t* test) increased migration only of RD/12 and RD/12-Neo cells, but not of RD/12-Myog and RD/18 cells expressing high myogenin levels. IL-4 receptor level (**B**) and signaling (**C**) in rhabdomyosarcoma cells with forced myogenin expression. Total proteins (100 μg) were loaded for the evaluation of total-STAT6, phospho-STAT6 (*STAT6 P*), IL-4 Rα, and IL-13 Rα expression. Actin (15 μg of total proteins) was used as a housekeeping protein for sample normalization. *M,* marker of molecular weight.

favored rhabdomyosarcoma cell growth and inhibited myogenin expression and myogenic differentiation.

Myogenin Expression Arrests Rhabdomyosarcoma Cell Migration

Muscle organogenesis requires migration of committed precursors followed by arrest, terminal differentiation, and somatic cell fusion to generate muscle fibers. Migration is also a fundamental determinant of invasive tumor growth and metastatic spread. Therefore, we studied the control of rhabdomyosarcoma cell movement by myogenin and IL-4. Poorly differentiated, myogenin-deficient RD/12 cells were more motile than RD/18 cells, whereas forced expression of myogenin significantly reduced cell motility (Fig. 4A). Chemotactic response to IL-4 was also drastically different; only myogenin-deficient RD/12 cells migrated toward IL-4, whereas both RD/18 and RD/12-Myog did not change their migratory behavior in response to IL-4. In conclusion, myogenin expression blocked both cell migration and IL-4–induced chemotaxis of human rhabdomyosarcoma cells.

To investigate whether myogenin expression directly modified IL-4 receptor level or activation, using RD/18, RD/12, and myogenin transfectants, we studied the expression of IL-4Rα and of its heterodimeric partner IL-13Rα (Fig. 4B), as well as the effects of IL-4 treatment on STAT6 phosphorylation (Fig. 4C). STAT6 activation was readily detectable after exposure of rhabdomyosarcoma cells to IL-4 (Fig. 4C). On the whole, neither IL-4 receptor levels nor activation state showed relevant differences in relation to myogenin expression.

Discussion

We showed that forced myogenin expression induces myogenic differentiation of human rhabdomyosarcoma and arrests cell migration, resulting in a significant inhibition of local tumor growth and metastatic spread to the liver. IL-4 was found to oppose myogenin expression and activity, favoring rhabdomyosarcoma cell growth and inhibiting myogenic differentiation.

The fundamental role of myogenic transcription factors such as MyoD and myogenin in controlling normal myogenic differentiation is well known (26). However, their activity in neoplastic myogenesis was taken for granted, because most rhabdomyosarcomas express myogenic factors and seem to be arrested at a later differentiation stage (27). We hypothesized that differences in myogenic propensity and in tumor malignancy between rhabdomyosarcoma clones (28) could result from the induction of myogenin expression (22). Here, we have shown that induction of a high myogenin expression is mechanistically related to a reduction in rhabdomyosarcoma malignancy, effectively identifying myogenin as a possible target of therapeutic endeavors, for example using gene therapy.

Myogenin simultaneously controlled the differentiation, replication, and migration of rhabdomyosarcoma. The former activities were expected on the basis of myogenin's role in normal myogenesis, whereas the inhibition of migration was not previously documented in rhabdomyosarcoma, and to the best of our knowledge, was only correlatively documented as a result of PAX3-FKHR silencing (29).

Myogenin-mediated activation of a coordinated myogenic program has manifold beneficial consequences in the control of neoplastic growth and spread *in vivo* because it simultaneously inhibits cell proliferation, induces terminal differentiation, and inhibits cell motility.

It is noteworthy that the control of neoplastic growth resulting from myogenin expression entailed a combination of cell-intrinsic and microenvironmental actions. In fact, local

tumor growth and liver metastasis were reduced by myogenin expression, whereas lung metastasization was not affected. We hypothesize that tumor growth and liver metastasis of rhabdomyosarcoma share a dependence from locally available growth factors, possibly including insulin-like growth factors and hepatocyte growth factor, which might be present only in traces in the lung microenvironment, thus explaining the very low incidence of lung metastases in our system. Our results suggest that the inhibition of cell growth and migration caused by myogenin could be mediated by modifications of growth factor receptors involved both in autocrine and in paracrine circuits (9–11, 13), a possibility that we are currently investigating.

The induction of differentiation and the inhibition of malignancy afforded by myogenin expression are far from complete. To further push rhabdomyosarcoma cells along the myogenic pathway, we tested IL-4, which in normal myogenic cells, was able to promote late phases of differentiation, in particular, cell fusion and maturation of myotubes which are defective in rhabdomyosarcoma (27).

In rhabdomyosarcoma, IL-4 enhanced cell growth, promoted migration of cells lacking myogenin and repressed myogenin expression, resulting in a coordinated repression of myogenic differentiation. The paradoxical effects of IL-4 on rhabdomyosarcoma could be reconciled with the physiologic activities of IL-4 by taking into consideration recent results showing that IL-4 is not required for fusion between mononuclear myoblasts, but for myotube maturation and also for myoblast migration (30). Therefore, IL-4 fosters normal myogenesis by stimulating the movement of precursors towards pre-existing myotubes and favoring myotube accretion through fusion with incoming myoblasts.

We do not need to postulate different responses of normal and neoplastic muscle cells to IL-4. In rhabdomyosarcoma, IL-4 promoted migration as it did in normal myoblasts. Other undesirable (from an oncologic point of view) effects of IL-4 are probably part of the coordinated actions of the cytokine on myogenesis. We found that IL-4 promoted cell growth at high cell density and inhibited apoptosis. Both activities could be instrumental in supporting growth and survival of myoblasts during muscle regeneration and possibly also in embryogenesis. Also, inhibition of myogenin expression (possibly via p38-MAPK/PI3K/AKT signaling circuits) could contribute to reducing the probability of myogenic fusion between myoblasts "in transit," to maximize the accretion of existing myotubes.

Unfortunately, the very low level of primary myotubes in rhabdomyosarcoma, and the protumoral consequences of supporting cell growth under saturating conditions and of inhibiting apoptosis, suggest that treatment of rhabdomyosarcoma with IL-4 would enhance tumor growth and malignancy. In rhabdomyosarcoma, it is clear that IL-4 promoting actions are exerted on differentiation-refractory cell populations, which contain cancer-initiating cells. It is interesting to note that IL-4 was also recently found to support the survival of cancer stem cells in colon carcinoma (31).

Forced myogenin expression made rhabdomyosarcoma cells resistant to IL-4–induced migration. This effect was not mediated by changes in IL-4 receptor level or activation. Myogenin-transduced rhabdomyosarcoma cells also showed a decreased metastatic ability to the liver, likely caused by microenvironmental factors. However, the involvement of murine liver–produced IL-4 can be ruled out based on the lack of cross-reactivity between the mouse cytokine and the human receptor. Microarray studies could allow a deeper analysis of changes caused by forced myogenin expression.

In conclusion, our results identify two new therapeutic targets to be attacked with opposite strategies in rhabdomyosarcoma. The induction of myogenin expression, possibly with gene therapy approaches, could enhance terminal differentiation and inhibit metastatic spread. Inhibition of IL-4, for example, with monoclonal antibodies or small molecules targeting the cytokine, its receptors and intracellular signal transducers, could simultaneously reduce cell survival in tumors and inhibit invasive and metastatic propensity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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