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Short Communication

Myogenic potential of mouse primordial germ cells

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ABSTRACT Primordial germ cells are the only stem cells that retain true developmental totipotency after gastrulation, express markers typical of totipotent/pluripotent status and are able both *in vivo* and *in vitro* to give rise to pluripotent stem cells as EC and EG cells. We have therefore explored the possibility of the trans-differentiation of mouse PGCs to a myogenic lineage by transplanting them directly or after *in vitro* culture into a regenerating muscle and by culturing them on monolayers of differentianting muscle cells. The results obtained suggest that mouse PGCs may trans-differentiate into myogenic cells, provided that their somatic environment is preserved. This occurs at an estimated frequency of 0.01%, which is no higher than that reported for stem cells of adult tissues.

KEY WORDS: *primordial germ cells, stem cells, myogenesis, transdifferentiation*

The concept that tissue-specific stem cells can give rise to cells of heterologous lineages has gained support from recent studies. For example: (1) bone marrow-derived cells can undergo myogenic differentiation (Ferrari *et al.,* 1998; Bittner *et al.,* 1999), can produce differentiated cell types in the brain (Mezey *et al.,* 2001) or form hepatocytes in the liver (Petersen *et al.,* 1999; Theise *et al.,* 2000); (2) neural stem cells can form blood-forming and muscle tissue (Bjonson *et al.,* 1999; Galli *et al.,* 2000) and a large variety of tissues when injected into early embryos (Clarke *et al.,* 2000); (3) hemopoietic stem cells can generate muscle, liver and multiple epithelial tissues (for a review, see Goodell, 2001); (4) skin stem cells can make neurons, glia, smooth muscle and adipocytes (Toma *et al.,* 2001).

Primordial germ cells (PGCs) are the embryonic precursors of the gametes of adult animals and are considered as the stem cells of the germline. Two important properties of PGCs are that they retain pluripotency (for a review, see Donovan and Gearhart, 2001) and possess a special capacity for epigenetic modifications of the genome (for a review, see Surani, 2001). Moreover, PGCs express markers typical of totipotent/pluripotent status, including the Oct-4 transcription factor (Yeom *et al.,* 1996; Nichols *et al.,* 1998), the gene *stella* (Saitou *et al.,* 2002) and KIT and LIF receptors (Matsui *et al.,* 1992; Cheng *et al.,* 1994). However, when mouse PGCs are introduced into a host blastocyst, they do not appear to contribute to either the germline or the soma, suggesting that they are normally restricted in developmental potency (McLaren and Durcova, 2001). On the other hand, when transplanted *in vivo*, PGCs give rise to pluripotent embryonal carcinoma (EC) cells that generate teratomas - benign tumors containing derivates of the three primary germ layers. Similarly, PGCs isolated from embryonic gonads onto feeder layers will, i n the presence of serum and certain growth factors, form pluripotent embryonic germ (EG) cells that are morphologically indistinguishable from EC cells or embryonic stem (ES) cells; these EG cells are capable of giving rise to somatic and germline chimeras (for a review, see Donovan, 1998). Collectively, these results suggest that PGC differentiation fate can be switched upon introduction into certain environments. If PGCs can be induced to exit the germ lineage and directly trans-differentiate into diverse cell types with a frequency higher than that of other apparently restricted stem cells (around 0.1 to 0.01%, for a review see Goodell, 2001), they could provide a relatively more accessible source of pluripotent stem cells for therapeutic use.

In the present paper, we have explored the potential of mouse PGCs to transdifferentiate into a myogenic lineage by culturing them on monolayers of differentiating muscle cells, or by transplanting them directly, or after expansion *in vitro*, into a regenerating muscle.

Serial tissue sections of tibialis anterior (TA) muscle 2 or 3 weeks after injection of total gonadal cells obtained from MLC1F/3F-nLacZ mice revealed fibers containing β-gal aligned nuclei (4

Abbreviations used in this paper: EC, embryonal carcinoma; EG, embryonic germ; ES, embryonic stem; PGC, primordial germ cell.

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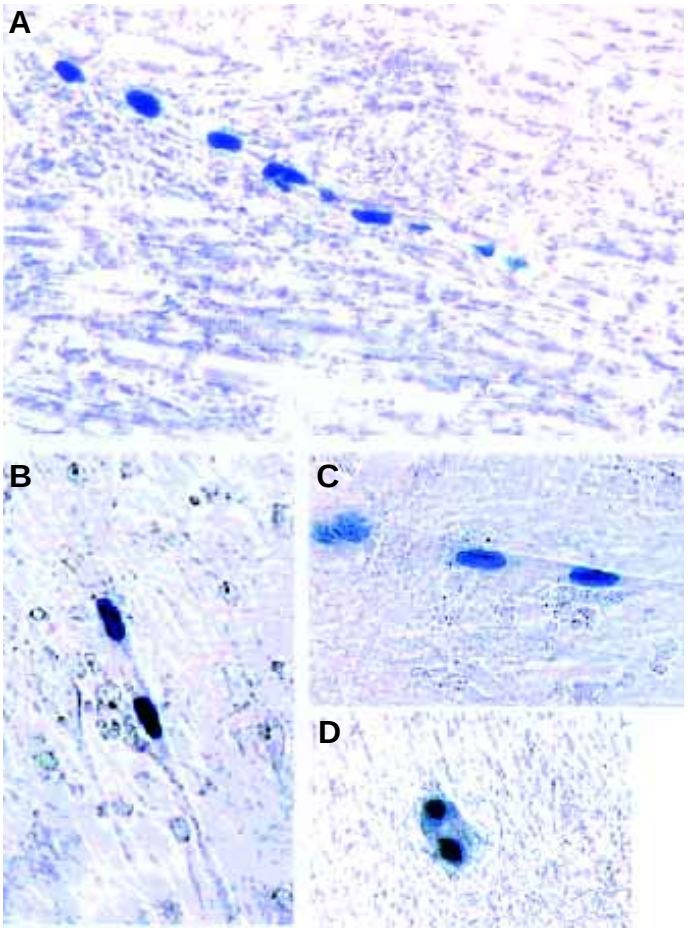

Fig. 1. (A) *Analysis of nuclear* lacZ *expression in cryostat sections of regenerating TA muscles from* scid/bg *mice transplanted with PGCs from* MLC1F/3F-nLacZ *mice. Muscles were analysed after 3 weeks. Longitudinal section: note a multinucleated myotube containing β-gal positive nuclei.* **(B-D)** *Examples of myotubes with β-gal positive nuclei present after 10 days of coculture of* MLC1F/3F-nLacZ *PGCs on C2C12 cell monolayers.*

out of 8 muscles examined) (Fig. 1A). Only sporadic single blue nuclei were observed in 2 out of 6 muscles injected with purified PGCs while no staining was observed in muscles injected with somatic cells. In the experiments in which TA muscles were injected with total gonadal cells cultured for 2-3 days on STO cell monolayers, the frequency of muscles containing β-gal positive cells was increased (5 out of 6). No blue nuclei were observed in 6 TA muscles injected with total gonadal cells cultured without forskolin (FRSK) and growth factors, or injected with purified cultured PGCs. Collectively these results indicate that in the total gonadal cell population there are cells with myogenic ability. β-gal positive fibers were found only when whole gonadal cell populations were injected, while only rare β-gal positive cells were found when purified PGCs were injected and none following somatic cell injection. This suggests that the myogenic cells originate from PGCs, but the PGCs need their gonadal somatic cells in order to survive and form myogenic cells in the regenerating muscle. This is also supported by the finding that β-gal positive cells were seen in TA muscles injected with PGCs from MLC1F/3F-nLacZ mice combined with somatic cells from wild type mice but not with somatic cells from blue mice combined with wild type PGCs (data not shown). The origin of β-gal positive cells from PGCs is also supported by the finding that the culture of total gonadal cells in the presence of FRSK and growth factors such as stem cell factor (SCF) and leukemia inhibitor factor (LIF), known to specifically favour PGC proliferation and transformation into EG cells in culture (for a review, see De Felici, 2001), increased the frequency of β-gal positive TA muscle to more than 80%.

The results obtained in the co-culture experiments of gonadal cells on C2C12 cell monolayers are also in line with such a hypothesis. We found that when total gonadal cells were co-cultured on C2C12 monolayers differentiating into myotubes, cells with β-gal positive nuclei were detectable after 7-10 days of culture (4 out of 4 dishes examined) (Fig. 1 B-D). In co-cultures in which purified PGCs (4 dishes) or somatic cells (4 dishes) were seeded, sporadic or no β-gal positive nuclei were seen, respectively.

In conclusion our results strongly suggest that mouse PGCs may trans-differentiate into myogenic cells provided that their somatic environment is preserved, and with an estimated frequency of 0.01%, comparable to that reported for stem cells of adult tissues (Goodell, 2001).

Experimental Procedures

PGC Isolation, Culture and Injection

Unless otherwise specified, PGCs and gonadal somatic cells were obtained from 11.5 days post coitum *(dpc)* embryos of the MLC1F/3F-nLacZ mouse strain, that expresses the *n-LacZ* reporter gene in nuclei of differentiated skeletal and cardiac muscle cells (Kelly *et al.,* 1995; Cossu *et al.,* 1995). PGC isolation and culture were performed as described previously (De Felici, 1998). Briefly, single cell suspensions of dissected gonadal ridges were prepared by EDTA-trypsin digestion, washed in Hepes-buffered MEM + 10% FCS (Gibco) and injected (about 1-2 $x10^5$ cells/leg, containing about 5-$10x10^3$ PGCs) into chemically damaged TA muscles of *scid/bg* mice (Charles River, Italy) (Ferrari *et al.,* 1998). In some experiments, PGCs (1-5 x 10^4 /leg, purity about 90%) and gonadal somatic cells (7-8 $x10^4$/leg, purity >95%) were purified using the MiniMACS immunomagnetic cell sorter (Pesce and De Felici, 1995) before injection. In other experiments, gonadal cell suspensions or purified PGCs were cultured in 5 cm Falcon tissue culture dishes containing mitomycin C-inactivated STO cell monolayers in DMEM (high glucose) plus 15% FCS (Gibco) with or without 10 μM FRSK (Sigma), 50 ng/ml SCF (R&D System) and 1000 UI/ml LIF (LIF_{ESGRO}, Gibco) (De Felici, 1998). After 2-3 days of culture, cells from 2 dishes were detached from the dish by trypsin-EDTA and injected into one leg.

Cryostat tissue sections of muscles were examined at 2-3 weeks from injection for the presence of β-gal positive nuclei (Ferrari *et al.,* 1998).

PGC Culture on C2C12 Cells

Gonadal cell suspensions (6-$8x10^4$ cells/dish, containing about 3-$4x10^3$ PGCs) or an equivalent number of purified PGCs and gonadal somatic cells were cultured in 5 cm Falcon tissue culture dishes on C2C12 cell monolayers. Cells were grown for 2-3 days in DMEM plus 15% FCS, with or without FRSK and the growth factors reported above. Then the cultures were shifted for an additional 7-10 days to DMEM containing only 2% horse serum (HS, Gibco), a condition permissive for C2C12 myogenic differentiation, and analysed for the presence of β-gal positive cells (Salvatori *et al.,* 1995).

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