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A unique method for the isolation of nasal olfactory stem cells in living rats

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Abstract Stem cells are attractive tools to develop new therapeutic strategies for a variety of disorders. While ethical and technical issues, associated with embryonic, fetal and neural stem cells, limit the translation to clinical applications, the nasal stem cells identified in the human olfactory mucosa stand as a promising candidate for stem cell-based therapies. Located in the back of the nose, this multipotent stem cell type is readily accessible in humans, a feature that makes these cells highly suitable for the development of autologous cell-based therapies. However, preclinical studies based on autologous transplantation of rodent olfactory stem cells are impeded because of the narrow opening of the nasal cavity. In this study, we report the development of a unique method permitting to quickly and safely biopsy olfactory mucosa in rats. Using this newly developed technique, rat stem cells expressing the stem cell marker Nestin were successfully isolated without requiring the sacrifice of the donor animal. As an evidence of the self-renewal capacity of the isolated cells, several millions of rat cells were amplified from a single biopsy within four weeks. Using an olfactory discrimination test, we additionally showed that this novel biopsy method does not affect the sense of smell and the learning and memory abilities of the operated animals. This study describes for the first time a methodology allowing the derivation of rat nasal cells in a way that is suitable for studying the effects of autologous transplantation of any cell type present in the olfactory mucosa in a wide variety of rat models.

Introduction

The use of stem cells for clinical applications represents a scientific challenge prompting innovative strategies and raising great hopes. To date, however, their use in clinic is rather limited to a few applications. Even though some bone, skin as well as corneal diseases or injuries can be treated with grafting of related tissues for which the success relies on stem cells present in the graft, hematopoietic stem cells remain the only stem cell type routinely used in the clinic. Indeed, hematopoietic stem cell transplantation allows for the treatment of diseases and conditions of the blood and immune system. Nevertheless, the plastic properties and the self-renewal capacities characterizing a stem cell raise ever

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growing hopes for the development of novel therapeutic strategies and have been proposed as a potential means of treatment for a variety of disorders (Brignier and Gewirtz, 2010; George, 2010; Kim and de Vellis, 2009; Lawall et al., 2010; Lindvall and Kokaia, 2006; Ronaghi et al., 2010). Despite the accumulation of data in favor of the utilization of stem cells and/or their derivatives, the translation from the bench to the bedside remains difficult to achieve. Thus, several problems such as ethical issues, cell availability, graft rejection and the risk of tumor formation can potentially be associated with their use (Boyd et al., 2012; Cantz and Martin, 2010; Li et al., 2008; Somoza and Rubio, 2012), which considerably curb their application to the clinic. Accordingly, the search for both stem cells and novel models of study allowing to bypass these constraints could contribute to speed up the long process leading to the development of new stem cell-based strategies. Along this line, the development of new methods involving autologous transplantations, when applicable, would represent a major advantage for the patients' safety and avoid several complications, placing this kind of research as one of the priorities. Nevertheless, animal models allowing the assessment of the effects induced by autologous stem cell transplantations are limited and often replaced by syngeneic transplantation instead.

Among the potential stem cell candidates for therapeutic development, we have highlighted the existence of a promising multipotent contender residing within the nasal olfactory mucosa (Delorme et al., 2010; Murrell et al., 2005; Tome et al., 2009), a peripheral and permanently self-renewing nervous tissue (Lindsay et al., 2010). This cell type has been characterized as a member of the mesenchymal stem cell superfamily displaying multilineage differentiation properties and a high proliferation rate in vitro (Delorme et al., 2010; Murrell et al., 2005; Tome et al., 2009). Of note, we coined the name "olfactory ecto-mesenchymal stem cells" (OE-MSCs) to define this new stem cell type (Delorme et al., 2010). Importantly, these cells present the advantage to be of ease access in human (Girard et al., 2011), which support their potential usefulness for autologous transplantation. In rodents, OE-MSCs have been successfully used in different models including myocardial infarct (McDonald et al., 2010), spinal cord trauma (Toft et al., 2012; Xiao et al., 2005, 2007), cochlear damage (Pandit et al., 2011) and Parkinson's disease (Murrell et al., 2008) as well as in a mouse model that mimics effects of ischemic/hypoxic injury in the hippocampus (Nivet et al., 2011).

In the present study, we focused our attention on olfactory lamina propria-derived stem cells. However, it can be pointed out that this tissue also harbors another cell type of great interest for the regenerative medicine, namely the olfactory ensheathing cells (Feron et al., 2005; Mackay-Sim et al., 2008).

Despite great promises raised by these different studies and the use of stem cells and/or ensheathing cells for clinical purposes, one major hurdle resides in the lack of a method allowing the isolation of these cells in living animals for testing the effects of autologous transplantations. Up to date, all data that have been accumulated rely on the use of syngeneic models (Bianco et al., 2004; Lu et al., 2001, 2002; McDonald et al., 2010; Toft et al., 2012) or xenotransplantations of human stem cells in rodent models (Murrell et al., 2008; Nivet et al., 2011; Pandit et al., 2011; Xiao et al., 2005, 2007) requiring in most cases the use of an immunosuppressant. Accordingly, the establishment of a method to harvest stem or ensheathing cells in an animal that could be at the same time the donor and the receiver of its own cells may represent a major advance to further test the full potential of these cells while excluding complications/side effects associated to the use of alternative methods of transplantation. While human olfactory mucosa is readily accessible in humans, allowing autologous transplantations, the narrow opening of the rodent nasal cavities can be seen as an impassable hurdle, preventing the possibility of cell autologous grafts in mice or rats. To overcome this problem, we developed a unique technique of olfactory mucosa excision allowing autologous grafts of nasal cells in rats. Biopsy collection, rodent nasal stem cell characterization and amplification as well as the consequence of the excision on the sense of smell and cognitive abilities of the donor animals are reported in this study.

Materials and methods

Rats

Ten-week-old male Sprague Dawley rats (n = 50; Charles River), at the beginning of the experiment, were used. All animals were housed in individual cages and maintained on a 12-hour light/12-hour dark cycle at a constant temperature (22 +/− 1 °C). Food and water were provided ad libitum except when tested for an olfactory associative discrimination task. Anesthesia and surgical procedures were performed according to the European law on Animal Care Guidelines, and the Animal Care Committee of Aix-Marseille University approved our protocols.

Excision of rat olfactory mucosa

Rat nasal olfactory mucosae were obtained by biopsies under anesthesia (Sodium pentobarbital, Nembutal, 60 mg/kg, ip) and under analgesia (buprenorphine hydrochloride, 0.03 mg/kg, paracetamol 4 mg/kg, ip). In a first set of experiments (n = 16), we tried to obtain pieces of olfactory mucosa by creating a 2 mm long and 1 mm wide lateral aperture (right or left) along the rostro-caudal axis, allowing a direct access to the olfactory mucosa. Then, in order to improve the success rate, we performed, in 34 rats, a 1 mm long and 2 mm wide medial aperture (on top the septum), allowing an excision on both sides of the nasal cavity (Fig. 1). More precisely, the head was first inserted in a stereotactic frame. After resection of the tissues covering the nasal and frontal bones at the level of the ocular globes, the window was drilled in the skull just anterior to the cribriform plate, 3.5 mm posterior to the nasofrontal suture and 9 mm anterior to the bregma, according to the rat brain atlas of Paxinos and Watson (2005). Using a "Hartmann Alligator Micro-Forceps" (model MCO13B, Microfrance, Medtronic), a 1 square millimeter biopsy was excised. This piece of olfactory mucosa was immediately transferred using a sterile needle into a sterile 2 mL tube filled with 37 °C DMEM/HAM's F12 culture medium (CM) supplemented with 10% Fetal Bovine Serum (FBS), 100 units/mL of penicillin and 100 μg/mL of streptomycin (Life Technologies). In order to exclude all risk of contamination, antibiotic concentrations can be doubled at 200 units/mL of penicillin and 200 μg/mL of streptomycin and 1.25 μg/mL of