Identification of the right cell sources for the production of therapeutically active extracellular vesicles in ischemic stroke

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Degenerative brain diseases including ischemic stroke result in the irreversible loss of brain tissue and are mostly associated with persistent neurological deficits. Commonly, the activity of endogenous stem and progenitor cells in these diseases is not sufficient to restore tissue homeostasis and neurological function. It is broadly assumed that the plasticity of endogenous stem and progenitor cells is insufficient in the adult brain to promote tissue remodeling and enable neurological recovery. Consequently, approaches were designed to treat such diseases with stem or progenitor cells assumed to have comparable developmental potentials than the endogenous stem cells.

At the turn of the millennium, when interest in stem cell biology increased exponentially, a number of observations implied plasticity in somatic stem cell compartments. Research results suggested that immature brain cells, under the right environmental conditions, are able to create blood cells and *vice versa* (1,2). In that area interest in fibroblastoid cells increased—in mesenchymal stem cells (MSCs)—that were raised from samples of adult bone marrow and showed multilineage differentiation capabilities (including bone, cartilage, fat, tendon, muscle, and bone marrow stroma) (3). In the following years, MSCs were raised from different tissues and tested for their developmental potential in various *in vitro* and *in vivo* assays. Many manuscripts reported developmental potentials far beyond that initially described by Pittenger and colleagues (3), e.g., that MSCs may directly differentiate into neurons (4).

Quickly MSCs emerged as a promising cell source in regenerative medicine and aside hematopoietic stem cells are now the 2nd most transplanted stem cell entity in NIH registered clinical trials. Although discussed controversially, MSCs have been found to improve the symptoms of several diseases, qualifying them as an important tool in regenerative medicine. Over the years, it became clear that against the initial assumption MSCs hardly intercalate into tissues. In several disease models their therapeutic effects have been associated with their immunomodulatory properties that were first reported in 2002 (5). It turned out that MSCs seem to act in a paracrine rather than in a cell-cell contact dependent manner (6). Furthermore, meanwhile their stem cell features have been challenged. Accordingly, many scientists nowadays prefer to call them mesenchymal stromal cells rather than mesenchymal stem cells. Recently, Arnold Caplan, a pioneer in the MSC field, recommended to name them even more precisely medicinal signaling cells (7).

Whatever the preferred terminus will be, trying to identify the active components exerting the MSCs' pro-regenerative/immunomodulatory activities, extracellular vesicles (EVs) have been identified to mediate the MSCs function in a variety of different disease models, including ischemic stroke (8-12). In ischemic stroke, systemically

administered EVs from MSCs induce neurological recovery via mechanisms that involved long-term neuroprotection, promotion of neurogenesis and angiogenesis, as well as reversal of post-ischemic immunodepression that is known to confer susceptibility to infection in the stroke recovery phase (12). Applied to a human GvHD patient, we showed that as in the ischemic stroke model EVs harvested from supernatants of human MSCs improved the GvHD symptoms and were able to modulate immune responses (12,13). Indeed, immunomodulatory features of MSC-EVs have meanwhile been described in several disease models, implying that immunomodulation is an important part of the MSC-EVs' proposed mode of actions that contributes to the pro-regenerative effects of MSC-EVs (14).

Apart of the MSCs, several other stem and progenitor types have been applied to animal models of various diseases. In ischemic stroke, we for example studied the impact of adult neural progenitor cells (NPCs), which were administered systemically or intracerebrally. If not immortalized, systemically administered adult NPCs were able to improve neurological deficits in contrast to intracerebrally administered ones (15). When systemically applied by intravenous delivery, the effects of adult NPCs strikingly resembled those of MSCs and MSC-EVs in a mouse model of ischemic stroke induced by intraluminal middle cerebral artery occlusion, involving prevention of very delayed post-ischemic neurodegeneration, reduction of brain leukocyte infiltration, reduction of astroglial scar formation and promotion of lesion-remote long distance axonal plasticity (16,17). Surprisingly, only a few systemically administered NPCs (0.1–0.3%) entered the ischemic brain, where most of them remained undifferentiated in the vicinity of the demarcating brain infarct (16). Based on these observations we already concluded that effects of NPCs are impossibly related to cell replacement. Indirect bystander effects, mediated by paracrine factors such as EVs, had to be involved. Aside MSCs and NPCs, there are other stem cell sources which successfully improved neurological recovery post-stroke, e.g., cells extracted from umbilical cord tissue or progenitor cells raised form induced pluripotent cells (18).

Apparently, independent of the type of the stem or progenitor cells administered (MSCs or NPCs), their administration seems to improve neurological recovery and brain remodeling in ischemic stroke. Intercalation and trans-differentiation of these cells into neural cells is usually not observed. The finding that EVs secreted by human embryonic stem (ES) cell-derived NPCs are able to improve neurological deficits and also modulate the stroke induced immune responses in a thromboembolic stroke model (19), implies parallels between the MSC-EVs' and NPC-EVs' mode of actions. Indeed, aside of EVs from both cell entities, EVs from several sources have been found to have immunomodulatory properties: starting with the fertilization process, immunomodulatory EVs, named prostasomes, have been found in the sperm liquids (20). Immunomodulatory EVs play essential roles during pregnancy (21) as well as in many developmental and regenerative processes and during tumor formation and expansion (22). Upon comparing the different systems, it becomes apparent that most of them are connected to developmental and regenerative processes and involve cell division. Suggestively, immune modulatory properties mediated by EVs are part of somatic stemness programs, implying tolerance inducing regulatory immune responses that are required to allow cell proliferation and successful tissue development or regeneration, respectively. In this context, it is worth mentioning that to our best knowledge all degenerative diseases including ischemia are associated with prolonged acute inflammatory responses. Against current dogmas, we came to the provoking hypothesis that at least a huge proportion of dividing cells and developing tissues are in principal immunogenic and are attacked if the immune system is in its acute inflammatory state. To allow development/regeneration we assume the immune system has to be switched from the acute inflammatory into the tolerance state. In this scenario endogenous stem and progenitor cells may contribute to the immune modulation by releasing tolerance inducing EVs, finally inducing an environment being permissive for tissue development and regeneration. If biased by pathogenic mechanisms towards its acute inflammatory state, the EVs from endogenous cells may not be sufficient to switch the immune response towards tolerance, resulting in a condition in which the inflamed tissue gets attacked. Accordingly, tissue remodeling is impaired. Upon administering somatic stem cells or their EVs, immunomodulation can occur and permissive environments for developmental and pro-regenerative processes are created, resulting in successful tissue remodeling. Notably, in good agreement with this hypothesis tumors effectively induce tolerance and suppress anti-tumor immune responses. Currently, it is a popular strategy in anti-tumor therapy adding check point inhibitors to switch the immune system from the tolerance state back to the acute inflammatory state (23). Thus, EVs from proliferating cells might be considered as tolerance inducing checkpoint activators.

As mentioned before at the example of ischemic stroke, in addition to their immunomodulatory activities, systemically administered EVs from MSCs induce neurological recovery by a combination of different mechanisms involving long-term neuroprotection, promotion of neurogenesis and angiogenesis. Accordingly, in addition to their immunomodulatory properties therapeutically active EVs may also induce other pro-regenerative processes being required to promote successful tissue regeneration. Mechanistically it has been demonstrated that for example MSC-EVs can increase ATP levels in damaged cells, reduce oxidative stress and the severity of cell injury, and restore cellular metabolic activities (24). For now, we do not know whether EVs from a certain stem cell type are better for certain applications than others and whether individual tissue have different, optimal EV source to stimulate regeneration.

It will be the goal for the next few years to identify the optimal EV source for each indication to be treated. In this context, there are several challenges connected to the field. Upon comparing the therapeutic potential of ES cell-derived NPC-EVs and ES cell-derived MSC-EVs in a murine thromboembolic mouse, NPC-EVs improved the symptoms much better than the MSC-EVs (19). However as already discussed by the authors, such results may not be representative for all MSC-EVs. For now, there is no agreed standard of how to prepare therapeutic EVs. Almost each group has their own strategy to prepare, characterize and analyze the prepared EVs' functional properties (14). As long as no reference material is internationally available or cross-lab comparisons are performed, it remains an open question, whether observed differences of one EV entity compared to the other one is due to lab specific protocols or whether EVs from a given cell entity indeed provide advantages in certain diseases than therapeutically active EVs from other cell types. Thus, it becomes important to constantly improve our criteria to define and systematically compare EV products. In this context, we are aiming to define in an international consortium, criteria for bona fide MSC-EVs and later eventually for other pro-regenerative acting EVs (10,25).

Independent, of whether certain therapeutically active EVs will be approved to be more potent in some diseases than others, we expect tolerance inducing EVs—especially those from stem and progenitor cells—may have huge clinical impact, especially when methods will have been optimized to produce EVs in scaled, GMP compliant manners.

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Footnote

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