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REVIEW

Risk of tumorigenicity in mesenchymal stromal cell–based therapies—Bridging scientific observations and regulatory viewpoints

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Abstract

In the past decade, the therapeutic value of mesenchymal stromal cells (MSCs) has been studied in various indications, thereby taking advantage of their immunosuppressive properties. Easy procurement from bone marrow, adipose tissue or other sources and conventional *in vitro* expansion culture have made their clinical use attractive. Bridging the gap between current scientific knowledge and regulatory prospects on the transformation potential and possible tumorigenicity of MSCs, the Cell Products Working Party and the Committee for Advanced Therapies organized a meeting with leading European experts in the field of MSCs. This meeting elucidated the risk of potential tumorigenicity related to MSC-based therapies from two angles: the scientific perspective and the regulatory point of view. The conclusions of this meeting, including the current regulatory thinking on quality, nonclinical and clinical aspects for MSCs, are presented in this review, leading to a clearer way forward for the development of such products.

Key Words: *cell, mesenchymal, stromal, tumorigenicity*

Introduction

Mesenchymal stem cells/mesenchymal stromal cells (MSCs) have been studied and used for more than a decade now to treat various diseases (1). Human MSCs are most commonly isolated from the mononuclear fraction of the bone marrow (BM) or from adipose tissue. Other sources for MSCs are also used, for example, some cell preparations are isolated from placenta, amniotic fluid or periosteum (2,3). The isolated MSCs show phenotypic heterogeneity, depending on the origin of the cells and the isolation/manufacturing techniques. According to current

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thinking, the cell surface proteins expressed by MSCs include cluster of differentiation (CD)105, CD73, CD44, CD90, CD71, Stro-1, CD106 CD166 and CD29. MSCs do not express hematopoietic markers such as CD45, CD14 or CD11. They also do not express co-stimulatory molecules CD80, CD86 or CD40 or adhesion molecules CD31, CD18 or CD56. In 2006, the research groups within the International Society for Stem Cells agreed on the minimal cell surface markers, considered necessary to distinguish mesenchymal stromal cells from other cell types (4).

Several researchers have described a role of MSCs in tumor formation (5,6). Furthermore, some groups have reported that human MSCs may develop genetic instabilities and undergo a transformation process after long-term culture, as suggested for adipose-derived MSCs (7), and for BM-derived MSCs (8). In contrast, however, several authors have reported that MSCs cultivated *in vitro* can be expanded over multiple cell doublings without apparent loss of differentiation potential or chromosomal alterations (9,10). In addition, long-term cultured MSCs can develop chromosomal abnormalities but without evidence of transformation potential (11). This discrepancy may be explained by reports that the cell cultures used in the studies performed by Rubio *et al.* (7) and Rosland *et al.* (8) were contaminated by malignant cells that initially grew slowly in the presence of human MSCs (12,13).

In October 2011, the Cell Products Working Party (CPWP) arranged an expert meeting to discuss the findings of the research groups working in this area. The expert meeting was intended to obtain information and discuss the opportunities and challenges currently faced when MSCs are used as therapeutic products. The focus was on tumorigenicity as a safety concern frequently expressed by regulatory authorities, although practicalities involved in the development of MSCs as medicinal product were also more broadly discussed.

Manufacturing and quality aspects

Garcia *et al.* (12) and Torsvik *et al.* (13) reported that the original observations of tumor formation for isolated MSCs were the result of MSC cultures contaminated with tumor cell lines. The meeting experts therefore emphasized the importance of recommending that cell culture is performed under good manufacturing practice conditions to ensure proper segregation and control of starting and raw materials.

The discussions also highlighted some specific issues around terminology and the need for harmonization. For example, the time for cells in culture is often described in terms of "cell passages." This was considered a nonspecific term that does not permit a suitable comparison for standardization purposes. Thus, the use of "population doubling level" (PDL) was unanimously recommended both by the experts and CPWP members. Concerning the impact of varying PDLs, it would be of interest to compare the different cell culture protocols used by different developers of a cell-based product for same indication (e.g., graft versus host disease [GvHD]). Overall, the maximum PDL for cell culture processes must be justified at the time of marketing authorization application.

The expert group discussed whether the manufacturing process could trigger the generation of cytogenetic abnormalities and what risk factors could promote tumorigenicity in MSCs. During the discussion, it appeared that culture conditions and duration of the cell propagation significantly influence the formation of cytogenetic abnormalities. Most experts considered that long-term cell expansion of MSCs may increase the risk of chromosomal aberrations. Some experts, however, did report that no such phenomenon had been seen when their own cell culture processes were used.

It was concluded that such abnormalities can be avoided through the use of methods that ensure slow growth and short expansion times because the number of expanded cells is linked to the growth rate, and a high proliferative rate may potentiate risk of karyotypic changes. A low PDL number was also considered an important factor in this regard.

It was also hypothesized that physiological stress or *in vitro* culture conditions may significantly contribute to the occurrence of cell or chromosomal aberrations: for example, enzymatic cell dissociation (i.e., trypsin) raises more concerns in relation to abnormalities than mechanical dissociation. Several reports indicate that culture conditions could also affect chromosomal stability (14,15). Therefore, it is important to identify and define culture conditions during process development, which avoid the occurrence of chromosomal abnormalities.

Cells with chromosomal aberrations are known to be less able to divide, which may lead to apoptosis and death of the abnormal cells during the culture (16). It appears that the majority of abnormalities lead to senescence, but it is difficult to formally exclude the risk of cell transformation because deoxyribonucleic acid (DNA) damage is considered a central process in tumor formation. It has been suggested by some experts that cell senescence and transformation could be evaluated with the use of certain molecular markers (11). However, this issue was not discussed in the meeting, and the original results remain to be confirmed. It is therefore important, for each defined processes, to force a sample of cells on an experimental basis into senescence through the use of