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Stem cells and cellular therapy / Cellules souches et thérapie cellulaire

New principles of cell plasticity

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Abstract – Recent discoveries demonstrating surprising cell plasticity in animals and humans call into question many long held assumptions regarding differentiative potential of adult cells. These assumptions reflect a classical paradigm of cell lineage development projected onto both prenatal development and post-natal maintenance and repair of tissues. The classical paradigm describes unidirectional, hierarchical lineages proceeding step-wise from totipotent or pluripotent stem cells through intermediate, ever more restricted progenitor cells, leading finally to 'terminally differentiated' cells. However, in light of both the recent discoveries and older clinical or experimental findings, we have suggested principles comprising a new paradigm of cell plasticity, summarized here. ***To cite this article: N.D. Theise, C. R. Biologies 325 (2002) 1039–1043.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

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Résumé – Plasticité cellulaire : nouvelles bases. Selon les conceptions classiques, le développement embryonnaire et la reconstitution des tissus adultes impliquent des lignages cellulaires hiérarchisés et unidirectionnels. Les mécanismes qui sous-tendent ces voies de différenciation sont présumés résulter de restrictions à l'activité des gènes, par exemple par méthylation ou formation d'hétérochromatine, couramment décrites comme irréversibles. Cependant, des découvertes récentes concernant des cellules souches multipotentielles démontrent qu'une « vraie plasticité » existe, des cellules d'un organe se transformant en cellules d'un autre organe, transgressant même les barrières entre les feuillets germinatifs primordiaux de l'embryon. Ces résultats, ainsi que ceux d'expériences plus anciennes sur la formation d'hétérokaryons et l'identification de longue date de lésions réactionnelles et néoplasiques chez l'Homme et l'animal, suggèrent que les voies de différenciation ne sont pas, en fait, unidirectionnelles. De plus, des mécanismes physiologiques assurant la réversion des restrictions géniques ont été identifiés. En conséquence, nous suggérons un nouveau paradigme de la plasticité cellulaire, guidé par trois principes. (*i*) Le génome doit être complet : toute cellule qui contient le génome entier, sans transpositions, ni multiplications, ni délétions, peut acquérir les caractéristiques de n'importe quel type cellulaire de l'organisme dont elle dérive. (*ii*) La caractérisation cellulaire est incertaine : toute tentative pour observer une cellule modifie l'état de cette cellule au moment de la caractérisation et peut donc modifier les possibilités d'événements de différenciation ultérieurs. (*iii*) L'origine des cellules et leur destinée est stochastique : la description des progéniteurs possibles ou de la progénie d'une cellule doit comporter les conditions d'observation et de manipulation du système observé et doit être exprimée sous forme stochastique, c'est-à-dire basée sur les probabilités. Ces principes impliquent un changement dans l'interprétation des données et la formulation des hypothèses. Ce nouveau paradigme nous conduira, espérons-le, à une exploration plus flexible et plus innovante du potentiel des cellules de Vertébrés adultes. ***Pour citer cet article : N.D. Theise, C. R. Biologies 325 (2002) 1039–1043.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

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1. Introduction

The classical paradigm of cell plasticity holds that there are cells in developing and post-natal tissues that actively maintain some degree of plasticity and those which have lost this capacity. These processes were elucidated by experiments where cells in a developing embryo were transplanted to new locations; such cells, which could first respond to the new environmental cues by expressing site-appropriate differentiation characteristics later seemed to lose this capacity. Subsequent investigations have highlighted dominant unidirectional, hierarchical lineage pathways in some adult tissues, most notably hematopoiesis [1], the germ line [2], and the gastrointestinal tract [3]. Thus it appears that plasticity is lost as development proceeds and in adult life. The molecular corollary to this loss is irreversible gene silencing which is tissue and cell-type specific [4, 5]. Such mechanisms of gene silencing have been identified, reinforcing the paradigm.

These gene-restrictive mechanisms act both by direct molecular modification of the genome as well as through so called 'epigenetic' mechanisms that modify the three dimensional structure of interphase chromatin resulting in genomic regions which are available for transcription (euchromatin) and those that are not available (heterochromatin) [6–8]. Methylation of cytosine residues located at the 5' side of guanine, often located in 'CpG islands' near the promoter site of genes, is the best-characterized mechanism involving direct modification of the genome [6, 9, 10]. This methylation may take place after epigenetic silencing of a gene by other mechanisms and serving to reinforce and perpetuate it [9]. Epigenetic mechanisms of gene restriction include methylation and/or deacetylation of histones by histone methyl-transferases and histone deacetylases, respectively [6, 7]. These events result in heterochromatin formation with tight coiling of the genome around the associated histones [7, 11]. Such tightly coiled regions are not available for transcriptional activation and are the last regions of the genome to be replicated during cell division [11]. In addition, these heterochromatin regions are commonly located at matrix attachment regions on the nuclear membrane and at the nucleolus, providing an exceptionally stable, if not rigid three-dimensional conformation [12, 13]. The euchromatic regions of the genome are simultaneously left open and mobile in the outer regions of the interphase chromosomal structure where they can more readily interact with transcription factors in the nucleoplasm [10, 12–13].

Suggestions that these gene restrictions are not necessarily irreversible, however, are to be found in several clinical and experimental observations from the recent and not so recent past. In adult tissues reactive metaplastic lesions have long been recognized [14]. Metaplasia usually confines the lineage changes to cell-types derived from the same original germ layer. In neoplasia, however, greater plasticity is often seen, for example in carcinosarcomas of many organs, in which clonal tumor cells variably display mesenchymal or epithelial differentiation [15].

Experimentally, heterokaryons, in which nuclear transfer from one terminally differentiated cell into another type of cell results in 'reprogramming' of the transferred nucleus [16, 17]. Thus, for example, a hepatocyte nucleus transferred into a myocyte begins to repress hepatocyte-specific gene expression and begin production of myocyte specific proteins [18]. This has led some researchers, most notably Helen Blau, to conclude that "differentiation requires continuous active control" by cell-specific cytoplasmic and nuclear factors [14, 19, 20]. Cloning, wherein a terminally differentiated somatic cell nucleus is completely reconditioned by the recipient oocyte, might perhaps be considered the most dramatic variant of this kind of experiment [21, 22].

All of these findings suggest that gene restrictions are not truly irreversible and careful examination of the recent literature shows that, indeed, mechanisms, both general and tissue specific, are being identified for the de-repression of 'silenced' genes. Active maintenance of silencing must take place, for example [23]. Gene methylation is reversed during embryogenesis [24] and in adult tissues [25] either passively during chromosomal replication [25], or actively via demethylase enzymes [25–27], or accomplished experimentally [28]. Demethylases for histones have also been identified, as have histone acetyl transferases that are capable of histone acetylation [6, 29–32].

To account for these clear demonstrations of differentiative potential lying outside the classical paradigm and bolstered by the increasing evidence that gene silencing is reversible we have suggested three new principles of cell plasticity [33, 34]. We feel that these principles are the logical extension of these new findings.

2. Three plasticity principles

2.1. Genomic completeness

Any cell that contains the entire genome – without deletions, duplications, or rearrangements – can become any other type of cell.

In the absence of irreversible gene restrictions there are no logical, conceptual limits on what cells can do.

Of course, in the body, there may be physiological limits, with pathways of greater and lesser probability, but theoretically any cell that has been isolated from the body and has an intact genome can be manipulated to become any other cell type. In fact, even with some specific gene deletions or rearrangements, there may be no limitations. In nature, adults with genetic defects [e.g. Gaucher's disease] are demonstrations of this [35]. Experimentally, reproductive cloning has been demonstrated using nuclei of mature lymphocytes that have already undergone gene rearrangement [36]. The limitations on cell differentiation seem to depend far more on the persistence and ingenuity of the experimenter than on the baseline state of the cell being manipulated [37].

2.2. Cellular uncertainty

Any act of observation, isolation, or characterization of a cell potentially alters the functional and differentiative capacity of that cell.

It is a truism that "the inside and the outside codetermine the cell" [38]. Thus, one may infer that to observe or otherwise interact with a cell necessarily changes the microenvironment and therefore changes the differentiation state or capacity of that cell. From the simplest act of venopuncture to more extreme acts of tissue disaggregation and culture, no investigation leaves a cell unchanged. The extraordinary complexity of molecular signaling pathways and of cell structure make this a certainty; to deny it is a useful reductionist approach, upon which nearly all our progress in cell biology has been based, but it is reductionist nonetheless.

This includes basic approaches to cell characterization that include antibody binding to cell surface molecules, which we often refer to as 'markers' as though they are merely name tags worn by the cell for our purposes. The activities of some markers, such as CD5 and CD45, have been extensively studied [39, 40]. It is clear that while some binding of ligand to these receptors can activate some cell processes, other forms of binding will produce alternate effects. So, before isolation with an anti-marker antibody can be assumed to be merely an isolation process, lacking influence on subsequent differentiation events, the relative inertness of the antibody binding needs to be established. If it has not been established then the interpretation of such data must take into consideration that possibility. However, most "markers" are not so well characterized and most do not have such a wide array of specific antibodies available for detection. A prime example of this is CD34: it still remains unclear what this molecule actually does [41], thus we have no way to determine what the sequelae of the use of detecting antibodies might actually be.

Whether this Uncertainty principle is merely a reflection of current technological limitations or is directly analogous to that of Heisenberg in quantum physics, an issue raised previously for example by Potten and Loeffler [42], is not yet clear. Is it possible to create a perfect machine by which a cell could be completely characterized, in situ, and yet remain unchanged? There is good reason to suggest that the answer is 'no', and that Uncertainty is ultimately irreducible, reflecting a fundamental aspect of cells and of our bodies.

2.3. Stochasticity of cell origin and fate

Descriptions of cell lineages, whether of descent or of destiny, must:
– be described in a stochastic – i.e. probability-based – manner;
– necessarily include the specific conditions of observation experimentation.

Combining the principles of Genomic Completeness and Cellular Uncertainty, one necessarily arrives at an understanding that while it may be a useful reductionist approach to treat cell differentiative processes as determined, they are actually stochastic in nature. For intact tissues and organisms, differentiative pathways are completely dependent on whether the tissue examined is physiologically normal or under stress of disease or injury. For isolated cells it is completely dependent on the method of isolation and the microenvironment to which they are exposed in culture.

Surprisingly, there is now some evidence to suggest that, like Uncertainty, this aspect of cells is not merely an artifact of technological limitations. Detailed studies of dynamic changes of chromosomal structure indicate entry points for randomness into gene expression and control – reviewed in [8]. One example: fluorescent labeling of euchromatin in the interphase nucleus reveals movement that is best modeled as a 'random walk' diffusion process, implying that interactions of genes with the important regulatory proteins in the spatially organized nucleosome, while tightly regulated in so many ways, also have an irreducible stochastic element [43].

3. Corollaries

Experimental outcomes that are small should not be dismissed as trivial, nor should outcomes, which fall short of 100%, be dismissed as merely 'contaminants'. While it is true that some techniques may result in unavoidable contaminants, this itself implies a stochas-

tic element. To dismiss these variations is to risk missing important, but subtle deviations from the more dominant physiological pathways or experimental potential of cells. Again, one is reminded of physics in the last century: the difference between Newtonian physics as opposed to Relativity lay in the former being a useful approximation that failed when confronted by the minute variations made detectable by advancing technology.

'Homogeneity' is an impossible ideal, no population of cells being completely identical across the scope of its genetic expression. Homogeneity as demonstrated by any set of markers at a given time point may not be stable even a moment after the characterization. The 'contaminants' mentioned above might be a reflection, not of technical limitations or a lack of precision, but of inherent reactivity and variability in gene expression in the population moment by moment. For example, just on the basis of cell cycles or circadian rhythms populations will contain variations [44]. Thus, one might say that homogeneity is "in the eye of the beholder", depending on the needs of the investigator. The question is not if a population is homogeneous, but if it is sufficiently homogeneous.

Failure to identify plasticity in a cell population should not be taken to imply that the sought after plasticity doesn't exist, merely that the technique to accomplish it has not yet been found. Conversely, the finding of plasticity in experimental conditions should not be presumed to reflect the functioning of the cell in vivo. In fact, it must always remain clear to the investigator that the cells maintained in culture are not the same as cells isolated from the original tissue, but are rather conditioned progeny of those cells. In essence, and perhaps most importantly: any act of isolation and characterization must be considered, simultaneously, a conditioning procedure.

4. Conclusion

These principles have the potential to cause both great excitement and great consternation to investigators. Consternation may arise because some aspects of the body, in particular the behavior and differentiative potential of any cell at any given moment, become unknowable in their details. Yet, there should also be great excitement because practical and therapeutic possibilities for cell manipulation and differentiation appear virtually unlimited, dependent only on our ingenuity and our persistence.

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