Statistical analysis of their results indicated sets of properties for each typical mass spectrometry method that predicted the propensity of a peptide to be detected with a high degree of accuracy. The study therefore confirms the widely held notion that different types of mass spectrometric approaches detect different segments of a proteome and describes physicochemical properties that can be used to score the likelihood of a peptide being observed. Validation of the approach, which was trained using yeast proteins, with a human data set confirmed that the strategy is generally applicable and should enable prediction of proteotypic peptides for any protein, irrespective of the availability of empirical data. However, as the analysis revealed no proteotypic peptides for ~40% of the proteins tested, it is unclear whether proteotypic peptides can be defined for all proteins, at least using current technologies.

Clearly, these approaches open exciting new avenues for analysis and cross-comparisons of quantitative proteomics data that do not require protein tagging or the use of internal calibrants. Working with yeast and *Escherichia coli*, Lu *et al.* measured absolute abundance that span more than three orders of magnitude and showed that they can reliably infer less than twofold differences in protein levels. They also showed that mRNA expression data are a good proxy for protein levels in the majority of cases (and at a log-scale). Furthermore, APEX data allows for the investigation of protein degradation rates and the systematic identification of unusual regulatory events indicated by extreme protein-mRNA expression ratios.

Computational prediction of proteotypic peptides, as described by Mallick *et al.*, will substantially expand the scope of proteomic discovery in species for which the full genomic complement has been characterized, but where limited experimentally-derived proteomic data are available.

Mass spectrometry–based peptide counting approaches that consider the proteotypic propensities of peptides will have far-reaching implications for experimental design. They open new avenues for computational improvements to peptide-identification software, and they may enable more-realistic assessments of the minimal set of peptides that are likely to be observed in mass spectrometry–based experiments and that collectively define a proteome—the proteotypic proteome. With such approaches—and the rapidly increasing sensitivity of mass spectrometry technology[8]—it should not be long before we arrive at the holy grail of proteomics: the discovery of disease biomarkers from patient biofluids.

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A fluid means of stem cell generation

Alan Trounson

Stem cells in amniotic fluid may represent an attractive alternative to embryonic and adult stem cells.

Pluripotentiality—the ability of a cell to form all the cells of the body—is generally considered to be confined to embryonic stem (ES) cells of the preimplantation embryo, embryonal carcinoma cells and embryonic germ cells of the primitive gonad[1]. Rare multipotential or pluripotential stem cells have also been isolated from cultured bone marrow cells[2] and spermatogonial cells of the testis[3]. In this issue, Atala and colleagues[4] describe a stem cell from another source, amniotic fluid, that can be directed into a wide range of cell types representing the three primary embryonic lineages of mesoderm, ectoderm and definitive endoderm (**Fig. 1**) If these results are confirmed by independent laboratories, amniotic fluid–derived stem (AFS) cells may become an important source of cells for regenerative medicine given their apparent advantages of accessibility and multipotentiality over embryonic and adult stem cells, respectively.

Amniotic fluid is known to contain a heterogeneous population of cell types derived from fetal tissues and the amnion. Atala and colleagues captured these cells from amniocentesis samples that were collected for prenatal genetic diagnosis of disease and chromosomal abnormalities, a procedure that is usually performed at 16–20 weeks of pregnancy. Normally, 10–20 ml of fluid is recovered, and the cell sample is divided into a test sample and back-up samples to be used if suitable preparations are not obtained from the original test sample. Using discarded back-up samples, the authors isolated AFS cells by selection for expression of the membrane stem cell factor receptor c-Kit, a common marker for multipotential stem cells.

AFS cells represent about 1% of the cells found in amniotic fluid. After a week of slow proliferation in culture, they adhere to plastic culture flasks and can be passaged at 70% confluence every two to three days. They have a high renewal capacity and can be expanded for over 250 doublings without any detectable loss of chromosomal telomere length. Moreover, the cells are not feeder-cell dependent and should be amenable to bulk culture for research and therapeutic applications.

Atala and colleagues show that AFS cells may be directed into a range of cell types with typical tissue characteristics. AFS cells can become nestin-positive neural stem cells, and then dopaminergic and glutamate-responsive neurons. AFS cell–derived neural stem cells grafted into the lateral cerebral ventricles of twitcher mutant mice dispersed throughout the brain parenchyma, preventing the distortion and neoplasia expected in these animals. In appropriate medium, the AFS cells also form functional osteoblasts that produce bone-like material when embedded in alginate/collagen scaffolds and grafted to immunodeficient mice. Other cell types were obtained, including putative hepatocytes capable of expressing liver proteins such as albumin, α-fetoprotein, hepatocyte nuclear factor and growth factor, and of secreting high levels of urea. These data are strongly indicative of the coordinated function of hepatic molecular pathways.

Although the origin of AFS cells has not yet been elucidated, it is likely that they derive from the amnion, a predictably attractive source of stem cells with multilineage

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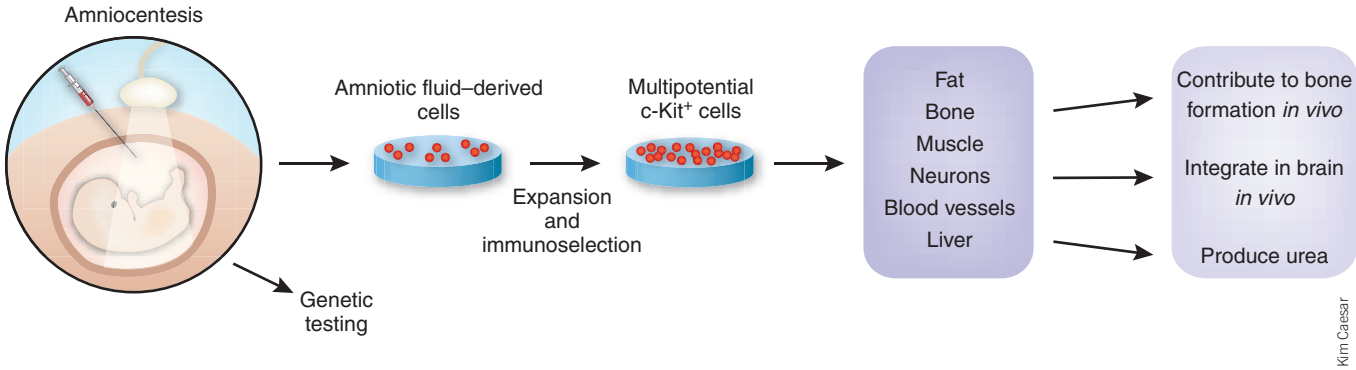

Figure 1 Multipotent amniotic fluid–derived stem cells are isolated by collecting a heterogenous population of cells through amniocentesis and selecting cells that express c-Kit. Under appropriate inducing conditions, the c-Kit+ cells can be differentiated into cells of all three primary germ layers.

potential[5–7]. The amnion, a membrane surrounding the fetus, arises from the epiblast of the differentiating blastocyst. The amniotic cavity forms between the embryonic ectoderm and mesoderm in the second week of human development[8], and persists throughout gestation. The epiblast origins of the amnion may be significant for the multipotential properties of AFS cells, and the amnion's longevity in gestation enables harvesting of these potentially valuable stem cells.

AFS cells express the pluripotential marker Oct4 and embryonic stage specific marker SSEA4, but not other markers of ES cells. They also express some markers of mesenchymal and neural stem cells, but not markers of hematopoietic stem cells. Unlike ES cells, they do not produce teratomas when transplanted to animals.

Multipotential c-Kit–positive cells isolated from dermis can acquire neural, hepatic and renal properties[9], and it is claimed that some c-Kit-positive cells from bone marrow have been differentiated into bone, cartilage, fat, neural and even pancreatic cell types[10]. It is apparent that AFS cells are different both from pluripotential ES cells and from multipotential adult stem cells, and may represent a new class of stem cells whose properties of plasticity exist somewhere between embryonic and adult stem cell types.

The availability of a renewable source of multipotential stem cells with the functional capacity to repair and regenerate a wide range of tissue types would be extremely valuable. AFS cells may approach the degree of plasticity of embryonic stem cells, and they could also be genetically manipulated. They are easily grown without feeder cells and, compared with less accessible cells of the early embryo, fetal gonads and testes, they are readily available from amniocentesis samples that would otherwise be discarded.

Potentially, AFS cells with a wide representation of histocompatibility types could be banked. The cells will come under close scrutiny for their immune-suppressive properties and their usefulness for transplantation in regenerative medicine. In their undifferentiated state, they may not be highly immunogenic because of their close association with maternal tissues in gestation. These factors could favor their use in allogenic transplantation and allow for a degree of mismatching without rejection.

The potential benefits and the obvious advantages of AFS cells may lead to a new wave of research to demonstrate the cells' utility in regenerative medicine and in other potential applications such as drug screening, tissue engineering and gene therapy.

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Evolving an anti-toxin antibody

Leonard Presta

The ability to engineer an antibody that specifically binds two epitopes shows promise for therapeutic and diagnostic applications.

The specificity with which monoclonal antibodies (mAbs) bind their targets is often acclaimed as the principal advantage of this class of therapeutics, as specificity likely reduces unwanted side effects. A study presented in this issue questions this truism, demonstrating that in some cases the utility of a mAb can be improved by expanding its binding repertoire. Garcia *et al.*[1] use yeast display and coselection to broaden the specificity of an antibody against botulinum neurotoxin type A1 to include the type A2 form of this neurotoxin, while maintaining picomolar affinity for type A1. The new antibody with cross-reactivity for both subtypes may have better therapeutic and/or diagnostic potential than its parent, albeit with some caveats discussed below. The authors also used structural analysis to determine the molecular basis of the broadened specificity.

As those of us in the Northern Hemisphere queue to get our influenza vaccine shots, it seems appropriate to reflect on the potential utility of passively administered mAbs directed against a variety of pathogens and

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