A striking finding of the human and mouse genome sequencing projects is that, although there are many differences between the two species, they have similar numbers of genes. The differences arise during development and are driven, in part, by changes in gene expression. The MRC–Wellcome Trust Human Developmental Biology Resource (HDBR) is a unique resource that provides human embryonic and foetal tissues to the scientific community, enabling gene-expression studies at these crucial periods of development.

Introduction

The human [1] and mouse genome [2] sequencing projects have produced an abundance of data and their analysis, although by no means complete, is revolutionizing molecular genetic and evolutionary studies (e.g. Ref. [3]). The data that have emerged on gene number in both species suggest fewer genes (~22 000) than were originally predicted (although problems remain with identifying non-coding RNA genes [4]). show similar numbers of predicted protein-coding genes and helped identify a human orthologue for 99% of mouse genes [2]. Given these findings, attention has refocused on the hypothesis that the temporo-spatial patterns (i.e. when and where
genes are expressed) rather than major differences in gene content shape the morphology and functional abilities of individual species (e.g. Ref. [5,6]).

Why study human development?
Many disorders are evident at birth and, even for disorders that appear later in life, it is now clear that many have their origins during foetal development (e.g. Ref. [7]). Determining the expression patterns during embryonic and foetal development of genes that underlie such disorders is, therefore, a crucial step towards revealing gene function. Clearly, it is also important to define the expression patterns of genes that regulate normal human development because this will help to reveal possible disease mechanisms, and might highlight potential direct or indirect targets for therapeutic interventions.

Not surprisingly, gene-expression patterns have been described in much more detail in model organisms, such as the mouse, than in humans. Several groups are undertaking large-scale gene-expression studies in mouse and they provide information on projects on laboratory-based websites (e.g. www.informatics.jax.org/mgihome/GXD/aboutGXD.shtml, genex.hgu.mrc.ac.uk/Emage/database/emageIntro.html, www.brainatlas.org/, www.nbirn.net/TestBeds/Mouse/index.htm, www.sanger.ac.uk/Teams/Team39/). Moreover, a range of sophisticated experimental techniques are also available for dissecting gene function in mouse and other animal species. Animal models, therefore, will continue to be relied on in studies of developmental mechanisms. Developing a rational basis for extrapolation from these model systems to human disease is important if we are to use the mouse (or other animal models) safely to investigate the mechanisms underlying human disorders and to evaluate possible therapies. There is already evidence that there are differences (e.g. Refs [8,9]) and similarities (e.g. Ref. [10]) in the expression of important developmental genes in humans compared with those of model organisms. In addition, certain mouse models mimic only part of the spectrum of the corresponding human disease (e.g. Refs [11,12]), and differences in basic aspects of mouse and human biology can have a significant impact on the effectiveness of potential therapies (e.g. Refs [13,14]).

Difficulties facing the analysis of gene expression during human development
Inherent difficulties in obtaining human embryonic and foetal material for research have hampered gene-expression studies in human development. Cultural and societal attitudes towards termination of pregnancy vary significantly between countries and, even where termination is practised, there are different legal frameworks defining the circumstances and stages of pregnancy when termination is permitted. In cases where abortus material can be obtained for analysis, it is unlikely to be available during the crucial period of early organogenesis (the third and fourth week of development), which is beyond the time period for in vitro fertilisation but, for practical reasons, before the period when terminations are usually performed.

Despite these difficulties in collecting human material, a few substantial archives do exist, in particular the Carnegie Collection [15]. Although enormously valuable for anatomical studies, this material is unsuitable for gene-expression studies (e.g. using tissue in situ hybridization), owing to RNA degradation following the standard collection and fixation methods employed.

Even where human material that is suitable for gene-expression studies can be collected, there are relatively few developmental biologists with experience in both gene-expression techniques and human developmental anatomy. Hence, the shortage and inaccessibility of human embryonic and foetal material, together with the lack of appropriate expertise for its interpretation, have seriously hampered human gene-expression studies to date.

HDBR – a unique resource
Responding to the need for human developmental gene-expression studies, the HDBR was established in 1999 to provide embryonic and early foetal tissue for research. HDBR material has already enabled studies of embryonic and foetal anatomy and gene-expression analysis, particularly by in situ hybridization and immunohistochemistry. It has also provided cells (including stem cells) for culture and biochemical analyses, and acted as a source of material for RNA isolation and characterization. The ongoing HDBR collection currently has >1000 specimens spanning the embryonic and early foetal period (4–12 weeks post-conception). Collection of material from pregnancy terminations is performed with appropriate written consent, approval from local ethics committees and in keeping with national guidelines [16].

A modified Carnegie staging system [15,17], based on macroscopic external morphological features, is used to group embryos into an ascending order of development (Figure 1a–d) up to the end of the embryonic period [Carnegie stage (CS) 23]. Beyond this stage, standard foot-length tables are used for the early foetal period. Only specimens that are morphologically normal (at least on external inspection) and have a normal karyotype are used routinely for gene-expression studies. As expected, given the known frequency of aneuploidy in first trimester pregnancies [18], we have detected an abnormal karyotype or visible abnormal morphology in ~10% of the samples. These abnormal embryos and foetuses will form the basis of future projects aimed at understanding the pathogenesis of developmental defects.

The HDBR is a unique resource and since its inception, human tissue has been distributed free of charge to registered users (Box 1), and it has already benefited many research projects (e.g. Refs [19–23]). The embryonic and foetal material is distributed either as paraffin-mounted sections or as fresh tissue. Rigorous quality controls are in place to ensure the tissue is stored optimally and to assess the quality of the RNA and tissue morphology (Figure 1i–l).

A new gene-expression service
In February 2004, an in situ hybridisation service was introduced (on a consumables-cost-recovery basis) to
facilitate gene-expression studies for users who do not have this technology available in their own laboratories. HDBR staff perform the gene-expression analysis on behalf of users, who provide cDNA probes specific for their gene of interest (for more information, see http://www.hdbr.org). Hence, registered users can gain access to human developmental tissue by whichever route is best suited to their needs: either analysis in their own laboratory or use of the in situ hybridization service. In this way, the most effective use is made of the limited and precious human embryonic and foetal material.

A new development at the HDBR is the use of optical projection tomography (OPT; Box 2) [24], a rapid, non-invasive technique for producing digital three-dimensional (3-D) models. This technique is currently being used to investigate the detailed internal morphology of embryonic specimens between ~4 and 8 weeks of development (CS12–CS23). The 3-D models provide a framework onto which gene expression and anatomy can be mapped to facilitate the interpretation of gene-expression patterns. Figure 1a–d and e–h shows example images from four stages of early development.

Figure 1. Stages of early human development. The upper panel shows four different stages of early human development and the middle panel shows volume-rendered images from 3-D OPT models at the same stages. (a) and (e) CS12 (~26 days post-conception); (b) and (f) CS14 (~32 days post-conception); (c) and (g) CS17 (~41 days post-conception); and (d) and (h) CS19 (~48 days post-conception). The lower panel (i–l) gives four examples of gene-expression patterns defined using probes that are used routinely as controls in tissue in situ hybridisation experiments, in parallel with experimental probes provided by HDBR-registered users. In examples i–k the signal is seen as a blue-purple deposit. Labelled cRNA probes were generated using digoxigenin-UTP (Roche Diagnostics) following the manufacturer’s instructions. In example l the signal is seen in white. In this example, the labelled cRNA probe was generated using 35S-UTP. Examples i–k show transverse sections at different levels at CS17. Example l shows a parasagittal section, at CS19. Probes from the following genes were used: (i) MLC2v – the signal is detected in the chambers of the heart; (j) SHH – the signal is detected in the floorplate of the spinal cord and the notochord (k); PAX6 – the signal is detected in the ventral spinal cord; (l) SOX9 – the signal is detected in the ventricular layer of the forebrain, midbrain, hind brain and in the spinal cord.
Box 1. A summary of the registration process and the conditions of use

Researchers who wish to use material from the HDBR should contact the Resource Manager (at hdbr@ncl.ac.uk or hdbr@ich.ucl.ac.uk) who will then send a registration form. The registration form asks for details of the proposed project, the experience of the user (if material is to be dispatched) and the institution where the researcher is based. The HDBR is overseen by a Joint Steering Committee, which has agreed that the following will be given priority:

(i) Research on a known disease gene, which is likely to have an informative expression pattern. Genes that are expected to have ubiquitous or widespread expression cannot be expected to have high priority.

(ii) Genes that are expected to be important in early development, and whose expression patterns might be informative (e.g. those genes producing interesting and relevant phenotypes in model organisms but not known to be disease genes).

(iii) Genes that can be expected to be important in human- (or primate-) specific functions (e.g. cognitive function and language).

(iv) Genes that have shown to be associated with significant anatomical, or functional, differences between mice and humans.

Material is only sent to users once evidence is provided of ethical approval for the project to be carried out from their local institute or other relevant ethics committee. Currently, we only send material within Europe. Once the project is registered, if users wish in situ hybridisation to be performed by the HDBR, then there is a second form to complete with more comprehensive technical details of the probes or antibodies for the study.

The conditions of use for the HDBR include agreement:

(i) To respect the value of this human material and ensure that it is used responsibly and only for the project agreed with the Joint Steering Committee.

(ii) That after the work is completed, images recorded and data analysed, the slides will be returned to the HDBR.

(iii) To make images available to the HDBR (once the user has published the data as required) so they can be incorporated into a data base that will be made generally available to the research community.

Future directions

A goal of the HDBR is to make results obtained with the human material widely accessible to the scientific community. Currently, results become available only when users publish their results in peer-reviewed journals. However, space for publication of data images is strictly limited in most journals. Mindful of this potential problem, the HDBR intends to create a web-accessible data base that, following publication and with users' permission, will make HDBR-derived data available to the entire scientific community. A prototype data base, based on the EMAGE data base (http://genex.hgu.mrc.ac.uk/Emage/database/emageIntro.html) should be available in 2006. It will be important in the future to have robust procedures, agreed with interested sectors of the scientific community, for editing and curating the data base that enable its development and expansion. Together with the increasing availability of expert assistance for gene-expression studies, through the in situ hybridisation service, this should permit a significant expansion in our knowledge of gene expression during early human development.

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References


Box 2. Optical projection tomography

Optical projection tomography (OPT; [24]) is a new, rapid and non-invasive 3-D modelling method that was developed by Dr. James Sharpe of the Edinburgh Mouse Atlas team [25] (http://genex.hgu.mrc.ac.uk/). It enables digital models to be generated from intact specimens made transparent using standard histological clearing agents. Images are captured of views through the whole specimen from different angles (400 views are taken to cover one 360° rotation) and computer software is then used to reconstruct the original 3-D information. The method has advantages of speed and good resolution even at early stages of development [26,27]. For example, OPT has the advantage over magnetic resonance imaging (MRI) in that detailed models can be produced from small samples. With MRI, low signal-to-noise ratios make it more difficult to obtain high quality data from embryos younger than CS17 [28]. MRI is likely to be more useful for specimens more advanced than CS23, where the size of the specimen and the density of the tissue are too great to enable penetration of the light. The computer-based models can be readily manipulated using MAPaint software (http://genex.hgu.mrc.ac.uk/Software/paint/).

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