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A DNA chip off the aging block

Microarray analysis of gene expression differences between young and aging fibroblasts may provide insight into the complex biological process of aging. But do changes in cultured cells from a few individuals accurately reflect overall changes in aging people?

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UNDERSTANDING THE MECHANISMS underlying the aging process remains one of the main unsolved problems of modern biology. Aging is a complex process involving many cell types, changing cell–cell interactions and in humans, long periods of study. Of necessity, most research on human aging has focused on model systems that age in a shorter time period and for which the environment is, more or less, under the investigator's control.

Some of the earliest and most influential experiments in models of human aging showed that serially cultured normal human fibroblasts had a decreasing replicative activity and were capable of undergoing only 50–60 rounds of cell division[1]. In addition, during their replicative life span, the cells maintained a normal karyotype and underwent morphologic and physiologic changes that were reminiscent of the changes that occur in some cells in the bodies of older people. Hayflick proposed that this senescence in culture is a model for human aging. The model has the advantages of a short lifetime (typically 3–6 months), a full complement of human genes and the investigator's ability to control the cellular environment. This approach has been somewhat controversial, however, because of a lack of definitive evidence relating replicative aging in culture to cellular aging *in vivo*.

In a recent *Science* article, Ly *et al*. examined the transcriptional profiles of fibroblast cultures taken from different age donors[2]. Using high-density oligonucleotode arrays to measure messenger RNA levels, they compared gene expression in cell cultures derived from a 7-year-old and a 9-year old, two 37-year-olds and three individuals in their 90s. The study also included three individuals with progeria (Hutchinson-Guildford syndrome, a so-called 'disease of precocious aging'): two 8-year-olds and one 9-year-old. They found that about 1% of the genes monitored showed reproducible expression level differences between different age samples, and that most of these genes were involved in mitosis and in extracellular matrix remodeling. The authors concluded that a central underlying process of aging involves errors in the mitotic machinery that lead to chromosomal pathologies and ultimately misregulation of essential structural, signaling and metabolic genes.

This study demonstrates the dazzling power of DNA microarray analysis to detect specific processes involved in the complex process of aging. However, despite the power of 'high through-put' technologies to show general patterns of gene expression, variations in gene expression among individuals must also be considered. The study of Ly *et al*. included only seven individuals as donors, two or three from each of three age groups, overlooking the enormous individual variability in humans and of cultured human cells[2]. The studies should be confirmed through the evaluation of a larger number of subjects of defined health status. Alternatively, one potential approach might be to 'pool' mRNA from 5–10 people from each age group to normalize for individual variation. Differences between age groups should also be compared with the individual differences within an age group.

The gene expression differences seen in patients with Hutchinson-Guilford syndrome should also be interpreted with caution. Individuals with this syndrome possess some characteristics common to normal aging, but not others. Thus, the disease cannot be considered a simple case of accelerated senescence. The control group of normal 7-year-old and 9-year old subjects, presumably chosen to match the ages of the progeroid donors, is also inappropriate for comparison with older individuals. In this case, it is difficult to determine whether differences in gene expression are associated with aging, or with specific events that occur during development. For example, the differences between 37-year olds and 90-year olds are not as great as the differences between adults and children.

Ly *et al*. sub-cultured actively dividing fibroblasts from human biopsies and compared gene expression between the different groups[2]. However amplification of cells in culture places them under immediate selection for those that are most actively replicating. Recent studies have shown that in cell cultures derived from healthy humans there is no correlation between donor age and replicative capacity, presumably reflecting this selection for the most actively replicating cells[4]. Thus, it is difficult to determine whether transcriptional differences in fibroblast cultures accurately reflect differences in aging humans.

It is surprising, then, that the most impressive of the gene expression differences reported by Ly *et al*. were associated with proliferative regulation[3]. It is essential in making comparisons of gene expression in fibroblasts that the proliferation status of individual cultures is well-controlled. It will also be necessary to analyze differences in gene expression of dermal cells taken directly from skin samples that have not been amplified in culture.

We now have at our disposal powerful tools that allow us to examine and dissect complex processes such as aging. Studies such as those by Ly *et al*. are pioneering efforts that will lead to a better understanding of the complex biology of aging as well as the limitations of the methodologies we use. Thoughtful use of these powerful techniques will lead us to better insights to the mechanisms of aging and the increasing vulnerability to disease which aging brings. Progress, however, will depend on a thorough understanding of the criteria of aging and the basic biology of the model systems, and an acknowledgement of strengths and limitations of these models.

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