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Keynote lecture: The genetics and epigenetics of altered proliferative homeostasis in ageing and cancer

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Abstract

Ageing mammals are subject to an amazing array of aberrations in proliferative homeostasis. These are of two basic types: the post-maturational failure to adequately replace effete somatic cells (atrophies) and excessive proliferations of somatic cells (hyperplasias). To a surprising degree, these occur side by side within the same tissues and are features of numerous mammalian geriatric disorders. Atrophy is the likely usual initial event, the proliferative response perhaps developing as a secondary, compensatory, initially adaptive reaction. We have little understanding of why this putative compensatory reaction so often fails to be appropriately regulated in ageing mammals, leading to such pathologies as chronic inflammation, fibrosis, metaplasia and neoplasia. Advances in formal genetic analysis, mutagenesis, stem cell biology and epigenetics are likely to provide major new understanding. Stochastic epigenetic shifts in gene expression are of growing interest, particularly in explaining intra-specific variations on rates and patterns of ageing. Nature may well have evolved such random fluctuations in gene expression as a type of group-selectionist adaptive strategy to cope with diverse stochastic environmental challenges. Alternatively, such background “noise” in transcription and translation may simply reflect a type of informational entropy.

Keywords

Biology of Ageing; Proliferative Homeostasis; Atrophy; Hyperplasia; Somatic cell mutation; Stem Cells; Genetic Analysis; Epigenetics

1. Some examples of altered proliferative homeostasis in ageing mammalian tissues

Table 1 [see (Martin 1979) for an earlier treatment] illustrates the wide variety of human geriatric pathologies that exhibit features of altered proliferative homeostasis, which can be defined as a failure to maintain an appropriate balance between cell loss and cell replacement. The results are regions of marked cell loss (tissue atrophy) and regions of inappropriate cell proliferation (hyperplasia). Pathologists often see these two aberrations side-by-side within the same tissues. Particularly common and widespread examples are tissues that lose specialized differentiated cells but also exhibit interstitial fibrosis. Another common example, seen in osteoarthritis, is the osteocytic proliferation (Heberden's node) associated with atrophy of the contiguous joint cartilage. Paradoxical juxtapositions of atrophy and hyperplasia can also affect the same cell type, however – for example regions of colonic mucosal hyperplasia mixed with

atrophic changes, endometrial hyperplasias within atrophic endometrium, basal cell papillomas within atrophic skin, etc.

2. Possible mechanisms underlying the loss of proliferative homeostasis during mammalian ageing

2.1 Nuclear DNA damage

Estimates of the range and quantities of DNA damage are sobering. These include single and double strand breaks, depurinations, depyrimidinations, cytosine deaminations, interstand cross links and numerous adducts, some known and probably others still unknown (reviewed, in part, by (Floyd1995;Lindahl1996). For example, Lindahl has estimated that a long lived human cell loses $\sim 10^4$ purines per day under *in vivo* conditions and several percent of its purine residues over the decades of a human life span (Lindahl1996). While there are some 150 DNA repair genes to deal with these challenges (Wood et al. 2005), somatic mutations do accumulate with age and are clearly associated with the emergence of malignant neoplasia. The quantitative aspects of such mutations can be striking. For the case of colon cancer, for example, there are estimates of 3.3×10^4 insertions, deletions and translocations in a typical cancer cell (Table 2). Such observations have led my colleague Lawrence A. Loeb to propose that a key to the development of malignancy is the emergence of a mutator phenotype (Loeb et al. 1974; Loeb2001). The rates of accumulation of point mutations in ageing non-neoplastic tissues have been measured for a few cell types. It is of interest that these rates appear to be higher for an epithelial cell type (renal tubular epithelial cells) as compared to a non-epithelial cell type (peripheral T lymphocytes) when a comparable assay is employed, consistent with the greater vulnerability of epithelial cells to oncogenesis (Martin et al. 1996).

2.2 Mitochondrial DNA damage

An important role for mitochondrial damage and dysfunction in the genesis of sarcopenia (atrophic changes of skeletal muscles) is becoming increasingly compelling. For example, mitochondrial deletions have been found to co-localize with electron transport abnormalities and these both are found in segmental regions of atrophy within single skeletal muscle fibres (Wanagat et al. 2001). A very wide range of mitochondrial mutations have also been found in microdissected colonic crypts (Taylor et al. 2003), consistent with a role in regional mucosal atrophy.

3. Epigenetic shifts in gene expression

I prefer the original use of the term “epinucleic” (Lederberg 1958) to “epigenetic”, but the latter has now become ubiquitous. In contrast to mutation, epigenetic alterations do not involve changes in the sequence of nucleotide base pairs or in the dosage or arrangement of genes. They involve chemical alterations superimposed upon the genome that serve to modulate gene expression. Current emphasis has been upon two broad chemical modifications: methylations of CpG islands within promoters and acetylations, deacetylations and methylations of the histone protein components of chromatin. The world of small RNA molecules is receiving a great deal more attention as epigenetic modulators, however. A particularly attractive concept is the notion that micro RNAs serve as “combinatorial rheostats” of gene expression (Bartel and Chen 2004).

Tom Kirkwood and Tuck Finch forcefully brought to our attention the importance of stochastic events in the biology of aging by writing a book on the subject (finch and kirkwood 2000). Recent experiments in the laboratory of Tom Johnson have highlighted the importance of stochastic events in the determination of life span in a model organism (*C. elegans*) amenable to seemingly complete control of variables attributable to genetic or environmental influences.

These workers could predict the life span of a one day old adult worm on the basis of the degree of expression of a reporter of a transgenic promoter of a heat shock gene following a transient heat shock. These phenotypes were not heritable, however, thus supporting stochastic variations in gene expression as the basis for what gerontologists have been observing for many decades – enormous variations of life span among members of genetically defined populations maintained under comparable conditions. Given the observations that transgenes tend to be unstable, it will be important to repeat these experiments using endogenous loci, perhaps using knockin technologies. The findings are consistent, however, with the epigenetic shifts in gene expression that are now being uncovered among human monozygotic twin pairs (Fraga et al. 2005; Haas et al. 2006).

It is interesting to speculate on the origins of these stochastic variations in gene expression. The *C. elegans* experiments, in particular, raise the possibility that some suitable range of random variations in gene expression may have been selected by nature to ensure the survival of a few individuals within populations facing legions of unpredictable environmental challenges. An alternative to this group selectionist hypothesis would be an unavoidable degree of background noise within complex interacting biochemical pathways – a sort of informational entropy (Shannon 1948a; Shannon 1948b).

How might stochastic shifts in gene expression account, to some degree, for the loss of proliferative homeostasis during ageing? There are some obvious candidates whose partial silencing or overexpression could mediate, in part, such phenotypes. They would include receptors for various paracrine and endocrine factors with impacts upon mitotic cell cycle function as well as structural and regulatory genes that control the expression of such mediators. Classical tumor suppressor genes and oncogenes would certainly be among loci of interest. It is by now quite clear, of course, that epigenetic mechanisms are of major importance in the alterations of mitotic cell function during oncogenesis, including the epigenetic silencing of my favorite locus, the Werner helicase/exonuclease (Agrelo et al. 2006). My favored scenario for the genesis of the loss of proliferative homeostasis during the aging of *Homo sapiens*, however, invokes atrophic changes as an initial event, the result of multi-focal clonal attenuation and replicative senescence of somatic cells, largely attributable to the loss of telomere repeats (Wright and Shay 2005). While the idea that this process was under natural selection as an adaptive buffer against cancer during the reproductive phases of the life cycle is attractive and widely accepted (Wright and Shay 2005; Rodier et al. 2005), perhaps a more powerful selective force for the emergence of clonal attenuation was the need to dampen organogenesis during embryonic development, during which time the process of tangential cell replication emerges from what can be assumed to be near-exponential stem cell types of replication (Martin 1993).

A number of my colleagues, including the late Vincent Cristofalo, to whom I dedicate this overview, have raised questions about the significance of replicative senescence, as observed *in vitro*, for ageing *in vivo* (Cristofalo et al. 1998; Lorenzini et al. 2005; Cristofalo 2005). My own view is that the weight of the evidence, too extensive and too controversial to permit a comprehensive critical review in this brief overview, is that clonal attenuation and replicative senescence does indeed occur *in vivo*. (I plan such a review for some future article.) A number of my own experiments establishing declines in the replicative potentials of cells of the arterial wall have been reviewed elsewhere (Martin 1987). Those experiments employed primary cloning and autoradiographic assays of explants. These methods are much more likely to reveal patterns of replicative potentials as they occur *in vivo*. It is perhaps not surprising that attempts to establish correlations between the replicative potentials of *established* mass cultures with donor age have not provided consistent support for an impact of aging, as these cultures sample only a very small subset of the cells that exist *in vivo*, possibly representing classes of adult progenitor stem cells. To reveal such phenotypes in these putative stem cells may require

superimposed environmental stresses, such as the hypoxia that occurs in postmortem tissues (Martin et al. 1970). A very recent study interrogated cells *in vivo* and has demonstrated markers of replicatively senescent cells in aging baboons, notably including markers of DNA damage, consistent with having been triggered by telomere loss (Herbig et al. 2006).

If atrophy is indeed the initial type of proliferative abnormality to emerge in ageing mammals, why do we also observe hyperplasias? A reasonable hypothesis, it seems to me, is that these proliferative foci arise as compensatory events. Alternatively, one can simply invoke stochastic, asynchronous variations in the rates of clonal attenuation among subsets of somatic cells that co-regulate their proliferative homeostasis. I proposed such a mechanism for the genesis of proliferative atherosclerotic lesions some years ago (Martin et al. 1975). In any event, families of proliferating somatic cells are surely subject to the vagaries of superimposed genetic and epigenetic events. Regardless of the major underlying mechanism for the paradoxical multi-focal hyperplasias one sees in ageing ¹tissues, they must contribute to tumor progression, a phenomenon that is part and parcel of carcinogenesis in all ageing mammals.

4. Stem cells

The dynamics of stem cell amplifications, their progressions to adult progenitor cells with more limited potentials and the ultimate terminal differentiations of subsets of their progeny must be fully understood if we are to be in a position, one day, to ameliorate age-related atrophies, hyperplasias and neoplasias. It is thus surprising that perhaps the most important question in stem cell biology has received virtually no attention from the biogerontological community[♥], namely the “immortal strand” hypothesis, as initially put forward by John Cairns (Cairns 1975). According to this hypothesis, when stem cells begin to divide asymmetrically, the chromosomes bearing the oldest template DNA are preferentially segregated to daughter cells that retain the properties of stem cells, thus minimizing the load of mutations in these valuable multipotent progenitor cells. Recent research has provided a degree of support for this idea (Potten et al. 2002; Rambhatla et al. 2005; Karpowicz et al. 2005). Stem cells, like most malignant cells and cells of the germ line, express telomerase and maintain telomere ends, thus avoiding premature exits from the cell cycle (Shay and Wright 2001).

5. Towards a formal genetic analysis of components of proliferative homeostasis

Some progress has been made in this direction. The genetic analysis of osteoporosis can serve as an example. Despite the likelihood of many loci, the majority of which can be expected to have only small effects, interesting candidate genes have been uncovered and are deserving of further investigation (Williams and Spector 2006; Huang and Kung 2006). These studies were made possible by the advent of dual energy X-Ray absorptiometry for the quantitation of bone mineralization (Mauck and Clarke 2006). Curiously lacking from these and other such investigations is the apparent lack of interest in uncovering alleles that provide extraordinary degrees of resistance to the phenotypes of interest (Martin 2002).

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[♥]The author was delighted to discover that, motivated by these introductory comments on this subject, Thomas Rando added to his excellent lecture on the biology of stem cells, recent unpublished research from his laboratory that supports the Cairns hypothesis.

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Table 1
Altered proliferative homeostasis in ageing *Homo sapiens*

Integument: epidermal atrophy, “liver spots”, seborrheic keratoses, basal cell ca, squamous cell ca, graying and loss of hair, eccrine sweat gland atrophy, apocrine sweat gland hyperplasia, stasis dermatitis, regional subcutaneous atrophy and hyperplasia
Sensory: lacrimal gland atrophy, corneal degenerations, cataractogenesis, age-related macular degeneration; presbycusis; olfactory loss
Musculo-Skeletal: sarcopenia, “fatty infiltration” of muscle, osteoarthritis, osteoporosis
Hematopoietic/Immune: anemias, myelodysplastic syndromes, leukemia, lymphoma, monoclonal gammopathy & multiple myeloma, autoimmune disorders (e.g., atrophic gastritis & polycythemia vera), immunosenescence (accelerated in AIDS)
CNS: reactive gliosis, dural and meningeal fibrosis
Cardiovascular: atherosclerosis, arteriosclerosis, myocardial interstitial fibrosis
Pulmonary: interstitial fibrosis, emphysema
Renal: glomerulosclerosis, interstitial fibrosis
Male Reproductive: benign prostatic hyperplasia (smooth muscle & glands), adenocarcinoma of prostate, testicular atrophy
Female Reproductive: ovarian atrophy and thecal cell hyperplasia, endometrial atrophy and hyperplasia, endometrial carcinoma, smooth muscle atrophy and leiomyomas of uterus
Endocrine: parenchymal atrophy with interstitial fibrosis, cell type-specific hyperplasias, adenomas
GI: mucosal and smooth muscle atrophy, hyperplastic polyps, adenomas and adenocarcinomas of colon and rectum

Table 2
Calculation of the total numbers of large-scale mutations (insertions, deletions and translocations) in a typical colon cancer cell¹ (Martin and Hu 2001) after (Stoler et al. 1999)

Ntμ = Total number “unique” inter-SSR PCR bands (insertions, deletions, and translocations) per typical colon cancer cell
N _{PCR} = Total number of alterations of all types, including changes in gene dosage, per set of PCRs
S _{PCR} = Total size of PCR fragments sampling the genome (base pairs)
H = Haploid genome size (base pairs)
N _{PCR-1} = Total number observed altered bands of all types
N _{PCR-μ} = Total number observed “unique” bands
P = Typical ploidy of a colon cancer cell
Ntμ = (N _{PCR} /S _{PCR}) × H × (N _{PCR-μ} /N _{PCR-1}) × P
Ntμ = (3/8.42 × 10 ⁴) × 3 × 10 ⁹ × (18/174) × ~3
Ntμ = 3.3 × 10 ⁴

¹ Such cancer cells typically have karyotypes that are near-triploid