Open-ended question: is immortality exclusively inherent to the germ line?

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Summary:

All somatic cells are subject to aging. Germ line links generations, and thus, pluripotent germ cells are considered potentially immortal. The current understanding how the germ line escapes this otherwise inevitable phenomenon is outlined in this article.

Back in the 19th century, the German cell biologist Friedrich Leopold August Weismann looked at primitive multicellular organisms such as Volvox. By tearing these delicate creatures apart, a cell type became apparent, which would immediately commence regrowth of a new Volvox. In view of this finding, Weismann proposed that germ line cells contain information that passes from generation to generation unaffected by the experience of the accompanying somatic cells [1]. These findings were further dissipated, and the assumption that genetic information cannot pass from the soma to the germ line and on to the next generation is now been referred to as the '*Weismann Barrier'*.

With some of his interpretations, Weissman was wrong (e.g. the *„germ-plasm theory"*), many however were simply brilliant. Besides putting forward the idea of an immortal germ line, Weismann proposed the concept that death of multicellular organisms is the result of worn-out tissues, which by means of cell division cannot renew themselves forever. Indeed, as an intrinsic property, human somatic cells senesce, and this was not noticed till the 1960's [2]. Cells derived from the inner cell mass of a blastocyst [3, 4] are bearing an intrinsic property, „the immortal germ line". Owing to their pluripotential differentiation capacity, they are also called embryonic stem cells (ES cells). In those cells, which are determined to form germ primordia and germ cells, the somatic program has to be properly repressed so that their pluripotent character is maintained. At that point the most pertinent question is whether pluripotency per se endows immortality?

The nature of germline was further specified in 1952, when Robert Briggs and Thomas J. King performed the first successful animal cloning experiment applying nuclear transfer technology. This proved the fact that like gametes also somatic cells contain the basic information needed to develop an adult individual [5]. Later on, John Gurdon pointed out that, albeit the cells' genetic potential for reprogramming is not diminished as the cell becomes specialized, only nuclei taken from an early embryonic stage were capable of developing fertile individuals [6]. This was but the first indication for distinct epigenetic changes becoming manifest during early development and the aging process. A most prominent example for the latter was the sheep Dolly, the first cloned mammal [7], for it developed arthritis at a rather young age of five years. A year later, Dolly had to be euthanized, because of progressive

lung disease [8]. It was already noticed that „young“ Dolly had shortened telomeres, which were inherited from the nuclear donor. In contrast to normal reproduction, correct telomere length re-equilibration apparently failed in this case [9].

Hence, the key issue raised by nuclear cloning was to unravel those mechanisms, which reside in the egg cytoplasm and cause the epigenetic conversion of the somatic program into the embryonic fate. Indeed „reprogramming factors“ could be unveiled. A combination of four genes, Oct4, Sox2, Klf4 and c-Myc turned out to sufficiently initiate reprogramming of differentiated cells into ES(-like) cells, also referred to as induced pluripotent stem cells (iPSC) [10]. This transition is indeed followed by distinct epigenetic changes, which are generally believed to determine a particular chromatin structure, the expression of regulatory RNA molecules, and a tightly knitted network of signal-transduction pathways, which altogether properly blaze the trail for reprogramming [11]. It is generally believed that germ cells employ equivalent mechanisms. In this context however, other circumstances have been appreciated to a much lesser extent: firstly the rate of reprogramming in cultured cells is astonishingly low. Interestingly though, cells of embryonic origin or tissue-specific stem cells reprogram more readily than terminally differentiated somatic cells do (reviewed in: [12]); furthermore, iPSC, although being competent to form germ line chimeras, give rise to animals that are prone to die from cancer already early in life [13].

Cell proliferation in renewable tissues puts the genome at risk for acquiring and propagating mutations. Hence, cancer afflicts complex organisms. In line with this, somatic maintenance evolved at the cost of certain tumor suppressor mechanisms. Some are thought to be involved in the aging process, be it for the repair of genomic damage, be it to stop propagation of potential cancer cells by permanently arresting their growth (senescence) or induction of cell death [14]. Needless to say, that such mechanism ought to be active in the germ line as well. It is not known whether as a consequence thereof, germline-associated cancers are rare. In the aging female hormonal imbalances, abnormalities in follicular development as a result of the aging of somatic cells surrounding the oocyte, or impaired perifollicular microcirculation become more common with advancing age [15]. Yet, it is generally accepted that besides follicular depletion at increased maternal age, anomalous oocytes are the

major cause for the decline in fertility [16]. In vitro fertilization studies have substantiated that oocyte donor age is the most important confounding factor [17]. How aging is affecting oocyte fitness, and thus directly diminishes the gamete's developmental potential and thereby impacts on immortality is still unknown.

After puberty of a male, spermatogonia continuously proliferate mitotically. Thereafter, daughter cells enter meiosis. In this way, sperm production is maintained through the entire lifetime. In aging men however, sperm fitness decreases, concomitantly, structural abnormalities in sperm increase [18]. Many chromosomal abnormalities such as the XYY karyotype or 45,X Turner's syndrome are of paternal origin. Yet, there is no clear relationship between paternal aging and the increase in the proportion of chromosomal abnormalities in their offsprings [19]. Characteristic alterations however are the reduction in seminal volume and sperm motility, but these are likely to be the results of age-associated changes of the function of the epidydimis, the prostate and the seminal vesicles. Apparently, aging greatly affects the reproductive system. Yet there is no good reason to believe that aging vitally impacts on germ line immortality. Hence at this point another question begs to be asked: do "reprogramming factors" act as a stimulant in a way to preserve germline cells young during ontogeny?

Primordial germ cell (PGC) become specified relatively late in embryonic development. Only a few of the pluripotent cells of the proximal-posterior epiblast, which express pluripotency-associated genes Oct4, Sox2, and Nanog, respond to the BMP-SMAD signaling that originates from extra-embryonic ectoderm and visceral endoderm. As a consequence thereof, PGC express Blimp1, which represses the incipient somatic program by preventing the loss of pluripotency [20]. What happens here is best exemplified by using the hen and egg concept: it is not that chicken lay more eggs to make more chickens; it is upside down: chickens are the eggs' way of making more eggs (Samuel Butler, 19th century); in other words, the soma is simply a transport vehicle. Yet it is highly unlikely that no molecular damage or cellular injury within the specified primordia, and later in development in germinal cells does occur. Hence, another question remains to be asked: what resets "age" in the germline?

How germline is protected is currently unknown. Well, unicellular organisms are also subjected to aging and thus may provide a rather simple model system to study how cells reset age. In budding yeast [21], mother and daughter cells are profoundly different. The mother devotes biosynthesis to the generation of daughters, and thereby ages. Buds are made young. At the future budding site, septins form a scaffold in the shape of a ring that rearranges into a collar at the mother-bud neck [22]. Many proteins bind asymmetrically to the septin collar [23]. Due to this septin-dependent diffusion barrier, aging factors, such as carbonylated proteins and DNA circles, remain confined to the aging mother cell when the cells divide asymmetrically [24]. Various types of stem cells are also known to undergo asymmetric cellular division. Every time a stem cell copies its chromosomes, it puts itself at risk of generating mutations in the new chromosomes. How stem cells manage to maintain genetic and epigenetic constancy throughout repeated divisions is poorly understood. One possibility is to segregate chromosomes asymmetrically, in other words, placing chromosomes with recently synthesized DNA strands into the daughter cell and keeping those with the original strand as an error-free, or „immortal“ template. This would minimize the risk to accumulate harmful genetic changes. This idea, called the "immortal strand hypothesis", was first proposed in 1975 by J. Cairns, as a mechanism for cells to reserve the mutation-free stability of their genomes [25]. In several stem cell types, there is experimental evidence for non-random segregation of chromosomes, These results indeed support the notion that stem cells follow the terms of Cairns’ theory [26-29]. What molecular mechanisms are applied by these particular cells in order to play this trick is currently unknown though. Besides that, it would be more than interesting whether immortal strands are what is inherited by the germ line.

Germ cells link the generations and are thus immortal. ESC, yet also adult tissue-specific cells such as multipotential adult progenitor cells, a subpopulation of mesenchymal stem cells [30] exhibit a high degree of pluripotency, and are therefore considered to have the potentiality for immortality. This leads us to the ultimate question, which remains to be answered by future developments in regenerative medicine: may we be able to prolong our existence through the use of pluripotent stem cells? [31]

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