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A HIERARCHICAL MODEL FOR THE CONTROL OF EPIGENETIC AGING IN MAMMALS

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ABSTRACT

Regulatory mechanisms range from a single level of control in simple metazoans to multi-level hierarchical control networks in higher animals. Organismal regulation encompasses homeostatic and circadian networks that are interconnected, with no documented exceptions. The epigenetic clock is a highly accurate biomarker of age in humans, defined by a mathematical algorithm based on the methylation of a subset of age-related CpG sites on DNA. Experimental evidence suggests the existence of an underlying regulatory mechanism. By analogy with other integrative systems as the neuroendocrine-immune network and the circadian clocks, a hierarchical organization in the control of the ticking rate of the epigenetic clock is hypothesized here. The hierarchical organization of the neuroendocrine, immune and circadian systems is briefly reviewed. This is followed by a brief review of the epigenetic clock at cell level. Finally, different lines of indirect evidence, consistent with the existence of a central pacemaker controlling the ticking rate of the epigenetic clock at organismal level are discussed. The concluding remarks put the hierarchical model proposed for the control of the clock into an evolutionary perspective. Within this perspective, the present hypothesis is intended as a conceptual outline based on designs consistently favored by evolution in higher animals.

1. Introductory Remarks

The multi-tissue age estimator also known as the epigenetic clock is a highly accurate biomarker of age in mammals, defined by a mathematical algorithm developed by S. Horvath (2013). Other epigenetic clocks have been devised (Bocklandt et al., 2011, Hannum et al., 2013, Weidner et al., 2014, Meer et al., 2018, Choi et al., 2019) but since they are all based on DNA methylation (DNAm) profiles, the control features to be discussed here for Horvath's clock will apply to all of them. During the 7 years elapsed after its publication, the validity of Horvath's clock has been demonstrated in humans and primates. So far, most studies on epigenetic aging have been epidemiologic, testing and uncovering links between pathologies, conditions, primary traits and other variables with epigenetic age acceleration. The remainder of studies on epigenetic aging was focused on looking into the mechanism underlying the clock (Horvath, 2013, Horvath and Raj, 2018, Horvath et al., 2018). Although the existence of a central synchronizer of epigenetic age in mammals is suggested by several lines of evidence to be reviewed here, such a central pacemaker has not, to our knowledge, been sought. The present article hypothesizes the existence of an organismal integrator of the rate of biological aging at peripheral level and proposes the brain as its most likely location. By analogy with other integrative systems as the neuroendocrine-immune network and the circadian clocks, a hierarchical organization in the control of the rate of the epigenetic clock is hypothesized.

2. Homeostasis In Higher Animals

Biological clocks can be considered part of the regulatory systems of higher animals. It therefore seems appropriate to present a brief introduction to another component of the regulatory systems namely, the homeostatic network.

Unicellular organisms and simple metazoans display only one level of homeostasis: the intracellular. In these systems, the cellular response to environmental challenges is coordinated at the level of DNA. Each cell functions with a great degree of autonomy in an unregulated environment. In contrast, higher organisms possess a hierarchical organization consisting of three levels of homeostasis: (a) intracellular homeostasis, which again is under the genomic control of each cell; (b) cell-to-cell communication, which constitutes a homeostatic mechanism at tissue level and represents an intermediate stage between intracellular and systemic homeostasis, and (c) homeostasis of the extracellular milieu, controlled by groups of specialized cells. In mammals, most of these specialized cells belong to the neuroendocrine and

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immune systems. As a whole, the neuroendocrine system controls the physical and chemical characteristics of the internal milieu (Toni, 2004). On its part the immune system, perceives, through antigenic recognition, an internal image of the macromolecular and cellular constituents of the body and reacts to particular distortions of the image. It can therefore be said that the immune system maintains the "biological" homeostasis of the organism. Experimental data clearly point to the existence of a complex immune-neuroendocrine network involving different cell types and structures which are capable of emitting and receiving signals bidirectionally (Besedovsky and Sorkin, 1977, Roszman and Brooks, 1988, Jankovic, 1989, Blom and Ottaviani, 2017). Under this perspective, the immune system appears not as a separate entity but as an integral part of an organismal homeostatic network. Epigenetics is deeply involved in the responsiveness of the neuroendocrine network to perturbations of the internal milieu (Zhang and Ho, 2011).

3. Neuroendocrine Control of Circadian Clocks

Circadian clocks constitute relevant evidence for a central role of the neuroendocrine system in the synchronization of physiology and behavior (Astiz et al., 2019). Thus, it is well-established that 24 -h rhythms are organized by a body-wide network of endogenous circadian clocks. Mammals possess a central pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) which integrates environmental light information to synchronize the neuroendocrine system and in turn all cells and tissues, to the external light-dark cycle (Mohawk et al., 2012, Berger, 2004, Yoo et al., 2004, Brown et al., 2008, Shaar and Sassone-Corsi, 2013). The pineal gland, which releases high levels of melatonin at night, also plays an integrative role in synchronizing various oscillators, especially in endocrine organs (Berger, 2004, Yoo et al., 2004, Brown et al., 2008, Shaar and Sassone-Corsi, 2013).

At the cellular level, the core circadian clock consists of an autoregulatory transcriptional-translational feedback loop involving the activators *Clock* and *Bmal1* and their target genes Per1, *Per2, Per3, Cry1*, and *Cry2,* whose gene products form a negative-feedback repressor complex (Mohawk et al., 2012, Berger, 2004, Yoo et al., 2004, Brown et al., 2008, Shaar and Sassone-Corsi, 2013).

When mouse cells are cultured, they initially retain circadian oscillations but the amplitude of these oscillations decreases rapidly. Furthermore, *ex vivo*, cells show independent tissue-specific circadian period and phases (Yoo et al., 2004). The same is true for human fibroblasts in culture (Brown et al., 2008), showing the relevance of neuroendocrine cues for a sustained organismal synchrony.

At epigenetic level, control of clock gene expression is mediated, at least in part, by DNA methylation and histone modifications (Shaar and Sassone-Corsi, 2013).

4. The Epigenetic Clock

4.1. DNA methylation as an accurate biological marker of age

It would appear that cells have a built-in pacemaker of biological age which seems to be driven by the epigenome. There is ample evidence that a major component of that epigenetic pacemaker is DNA methylation. The first clear indication that DNA methylation is a major component of this pacemaker came with a 2013 report by S. Horvath demonstrating that the measurement of 353 particular methylation sites, known as cytosine-guanine dinucleotides (CpG), allows in humans, a highly accurate estimate of chronological age that applies to virtually all human tissues and cell types (Horvath, 2013). The measurement is performed by feeding a mathematical algorithm with the % methylation (beta value) of each of the 353 age-dependent methylation CpGs. The algorithm is known as the pan tissue epigenetic clock, also called the Horvath clock. The correlation between the epigenetic age estimate and chronological age exceeds 0.95 in data sets comprised of children and old people which shows that the epigenetic clock is arguably the most accurate molecular biomarker of age (Horvath, 2013, Horvath and Raj, 2018, Raj, 2018).

Since in most, if not all, animal species biological age changes with the passage of time so does epigenetic age (Horvath, 2013). The change in epigenetic age per unit of time is defined as the ticking of the epigenetic clock and it is presently unclear what constitutes its ticking in each tissue. It has been proposed that the ticking rate may be represented by the methylation changes that accompany the differentiation of tissue stem cells or that the ticking rate reflects the actions of an epigenomic maintenance system (Horvath and Raj, 2018, Raj and Horvath, 2020).

4.2. Control of the epigenetic clock at organismal level

Although there is evidence suggesting that the cellular epigenetic clock possesses an intrinsic ticking rate (Hoshino et al., 2019, Weidner et al., 2015, Søraas et al., 2019), multiple observations at organismal level in humans and other mammals lead to the inference that *in vivo*, the ticking rate of the clock in tissues is synchronized by a master pacemaker.

Considering again the genesis of the epigenetic clock, it is of interest to mention that in 2013 Horvath´s algorithm was successfully tested using approximately 8,000 DNA methylation data sets from over 30 different tissue types (Horvath, 2013). For a given chronological age, it was found that in DNA samples taken from whole blood, peripheral blood mononuclear cells, buccal epithelium, colon, adipose, liver, lung, saliva, and uterine cervix, Horvath's algorithm read essentially the same epigenetic age, the only exceptions being some brain regions and very few other organs (Horvath, 2013, Horvath et al., 2018). The same is true for mice (Meer et al., 2018; Thompson et al., 2019). Additional evidence comes from two other studies, one showing that DNAm over specific Polycomb Repressor Complex-2 (PRC2) promoter loci correlates with age across many different tissue-types (Teschendorff et al., 2010), and from another report in which a comprehensive statistical analysis, including matched multi cell-type and multi-tissue DNA methylation profiles from the same individuals, adjusting for cell-type heterogeneity, showed that a substantial proportion (possibly over 70%) of epigenetic drift is shared among significant numbers of different tissue/cell types (Zhu et al., 2018). The observed synchronicity that exists in the organs of mammals despite the very different proliferation dynamics among certain tissues, points to the influence of a master synchronizer.

In his 2013 paper, Horvath demonstrated that in humans, changes to the methylation rate of the 353 CpGs can be fitted to two different phases that are separated by a nonlinear transition rate. The first linear phase takes place from birth to about 1 year of age, where the slope of the fitted line is exceedingly steep. Then, a nonlinear phase occurs from about 1 year to about 20 years of age, where the rate of change in epigenetic age decreases. A third and final linear phase starts from 20 years of age onward, but in this case the slope of the line is much lower than that of the initial linear phase (Horvath, 2013, Raj, 2018). Since it seems highly unlikely that body cells in culture would follow such a three-phase long-term dynamics in the ticking rate of the epigenetic clock, we are again led to the hypothesis that a central synchronizer is controlling the developmental changes in the rate of epigenetic aging at organismal level. Therefore, it seems likely that as in the case of homeostasis and circadian clocks, epigenetic age may also be hierarchically controlled.

By analogy with homeostasis and circadian clocks, it seems reasonable to expect that organismal epigenetic aging be synchronized by specialized groups of command cells located in the brain, more specifically in the hypothalamus and higher brain centers modulating hypothalamic function (Fig. 1).

In closing this section it is important to mention a recent preliminary report in rats that may shed considerable light on the existence

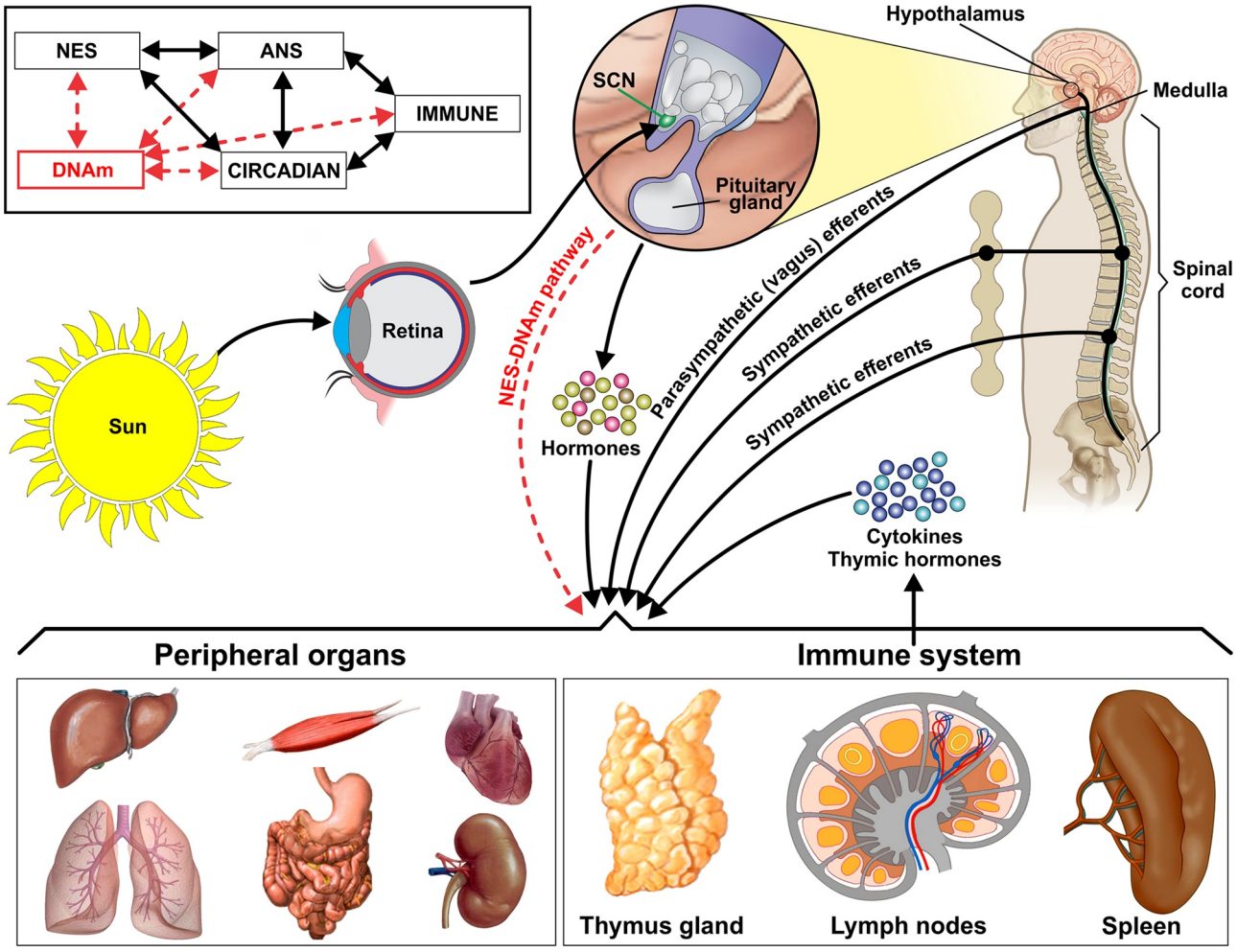

Fig. 1. Proposed organismal regulatory network in mammals. The diagram includes the autonomic nervous system (ANS, acting via neurotransmitters), the neuroendocrine system (NES, acting via blood-borne hormones), the immune system (acting via blood-borne cytokines and thymic hormones), the circadian clocks (acting via blood-borne hormones and neurotransmitters) and a putative pathway connecting the neuroendocrine network to the DNAm clock in organs and cells. All networks act on peripheral organs. **Inset-** Bidirectional interactions among all networks including (in red) the hypothetical DNAm network.
References: SCN, suprachiasmatic nucleus; DNAm, DNA methylation.

of a central pacemaker controlling epigenetic age. The study reports, first, the setting up of an epigenetic clock for rats including the design of a pan tissue epigenetic clock for this species. Using this new tool, the rate of epigenetic aging of peripheral tissues including liver, ovary, skin, adipose tissue and blood are shown to be similar like in humans and mice (Horvath, 2013, Meer et al., 2018; Thompson et al., 2018). The rate of epigenetic aging of a number of brain regions, including the neocortex, cerebellum, hippocampus, substantia nigra, hypothalamus and anterior pituitary were also assessed. The second part of this study is perhaps the most significant. It reports that repeated intravenous administration of a plasma fraction (termed Elixir) from young rats to old counterparts during 5 months, sets back the epigenetic age of liver, blood and heart tissue of the treated old rats (25 months old) to nearly that of adult rats (7 months old). The effect of Elixir on the DNAm clock was paralleled by significant functional improvements in a number of hematological, biochemical and functional parameters. The only exception was the hypothalamus where Elixir showed a modest although still significant, rejuvenation effect on DNAm age (Horvath et al., 2020).

The implications of this report, if confirmed, will be highly revealing. It suggests that blood-borne factors from young rats possess strong rejuvenation effects on the epigenetic clock of both blood and non-blood tissues from old rats. This is consistent with the idea that a central pacemaker could control the ticking rate of the epigenetic clock of peripheral tissues via blood-borne factors, a mechanism used by other regulatory centers of higher animals. Furthermore, the limited responsiveness of the DNAm clock of the hypothalamus to the regulatory factors present in Elixir is consistent with the idea that the slow rate of epigenetic aging observed in the hypothalamus of rats may be due to a relative insensitivity of this brain region to endogenous blood-borne regulatory factors secreted by the central pacemaker of the animals.

5. Concluding Remarks

Evolution of biological systems is associated with an increasing complexity at every level. Regulatory mechanisms range from a single level of control, in very simple systems, to multi-level hierarchical control networks in higher animals. In the latter, organismal regulation encompasses homeostatic and pacemaker networks that are interconnected (Fig. 1). The epigenetic clock concept emerged from a mathematical algorithm as a biomarker of age but experiments soon suggested the presence of an underlying regulatory mechanism associated to methylation of a subset of age-related CpG sites on DNA. The search for the mechanism that drives the clock is attracting an increasing number of scientists from multiple areas of biomedical research who in general, focus their work on cells and tissues. The eventual identification of a hierarchical control mechanism at

organismal level would strengthen the evidence for a common evolutionary pattern for the design of regulatory systems that emerged as simpler versions in primitive animals, becoming highly sophisticated in more evolutionarily recent animal species, particularly mammals.

The well-established regulatory systems reviewed above are expected to provide a frame of reference to guide the quest for an analogous regulatory pattern for the epigenetic clock. Within this frame of reference, the hypothesis proposed here is intended as a conceptual outline based on designs consistently favored by evolution in higher animals.

Consent for publication

All authors grant their consent for publication of this article.

Authors' contributions

The bibliographic search was equitably distributed among all authors. Each author prepared a draft of an assigned section. RGG was in charge of writing the final version. All authors read and approved the final version.

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Ethics approval and consent to participate

All authors agree to publish this article and have accepted to abide by the ethical standards of our Institution.

Availability of data and material

The information reviewed here are public domain.

Declaration of Competing Interest

None of the authors has competing interests.

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