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REVIEW

Epididymosomes: the black box of Darwin's pangenesis?

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ABSTRACT: Darwin, in the pangenesis theory, imagined particles, named as 'gemmules', which are released from all ('pan') cells of the body. By cell–cell communication and also circulation through the body, they finally reach the germ cells to participate in the generation ('genesis') of the new individual. It has been shown that circulatory exosomes are affected by environmental stressors and they can reach the parental germ cells. Therefore, in the mirror of his theory, circulatory exosomes could interact with epididymosomes: epididymis-derived exosomes which have a wide spectrum of variation in content and size, are very sensitive to environmental stressors, and may be involved in translating external information to the germ cells. The protein and RNA cargo would be transferred by epididymosomes to sperm during sperm maturation, which would be then delivered to the embryo at fertilization and inherited by offspring. Therefore, in this study, we will briefly discuss Darwin's pangenesis theory and its possible relation with epididymosomes. We believed that epididymosomes could be considered as an attractive candidate for the storage of RNA contents, changing the epigenome of the next generations, and allowing the reappearance acquired characteristics of ancestors. Therefore, epididymosomes, as a black box of Darwin's pangenesis, may unravel parental life history and also disclose the historical events that affect the life of offspring.

Key words: acquired characteristics / epididymosomes / Lamarck / Darwin's pangenesis / black box

Introduction

According to Hippocrates' hypothesis, 'Semen contains all parts of a human body and is secreted from a father's healthy and unhealthy organs to produce healthy or unhealthy parts in the child' (Adams, 1886). Similarly, 'Jerome Cardan (1501–1576) concluded that the semen was derived from the whole body' (Zirkle, 1935). These notable quotations were a little strange as well as very astonishing in that period of time. Moreover, scientific translation of this expression in modern biology could be associated with epididymosomes, epididymis-derived exosomes, which could be an attractive candidate for studying the composition of semen, due to the transfer of a wide spectrum of molecules from these exosomes to sperm during the maturation process and the subsequent reflection of such alterations in the succeeding generations.

A great deal of attention was paid to Hippocrates' opinion in the late 17th and early 18th century due to the growth of the experimental sciences (Mayr, 1982). In this regard, Erasmus Darwin, Charles Darwin's grandfather, in his book 'Zoonomia; or the Laws of Organic Life' discussed the parental particles, which are altered by their activities and environmental effectors, and could be then transferred to the future generations (Darwin, 1809).

Erasmus' theory is similar to Lamarck's theory of acquired characteristics (ACs), which are known as definite alterations obtained by living organisms during their developmental process in response to specific environmental stressors (Tikhodeyev, 2020). In the early 18th century, Jean-Baptiste Lamarck presented the first evidence in terms of the hereditability of ACs in his book entitled 'Zoological Philosophy' (de Monet, 1809). Accordingly, he stressed that the indefinite variations are non-heritable and only if the effect of definite variations is lasting, it could lead to heritable alterations (de Monet, 1809). After the introduction of Lamarck's theory, which was dependent on the decisive role of the environment and also on the preservation of the changes caused by reproduction in the succeeding generations, several critical ambiguities were raised in terms of the transformation of the environmental information to the germ cells (Lamarck, 1914). Conversely, August Weismann strongly disagreed with ACs, believing that parental germ cells are responsible for the transmission of genetic information to progenies (Weismann *et al.*, 1891). Moreover, he stated that the germ cells are protected from the influence of the environmental stressors as there is preservation of germ cells against environmental alterations (Weismann *et al.*, 1891). However, other scientists started to conduct a growing body of experiments to assess Lamarck's theory as their efforts were focused on the possible mode of actions during the transmission of environmental information to the germ cells (Aucamp *et al.*, 2016). Accordingly, these studies, known as 'somatic induction' studies, showed that there is some evidence that supports inheritance of acquired characteristics (IACs), and also rejected the germ-plasm theory (Aucamp *et al.*, 2016).

Charles Darwin was inspired by Hippocrates and Lamarck and aimed to explain how variation took place in heredity (in other words, how IAC would occur) (Aucamp *et al.*, 2016). Darwin's Pangenesis refers to the imagination of the particles, named as 'gemmules', released from all ('pan') cells of the body (Darwin, 1868), which by cell–cell communication and also circulation through the body, finally reach the germ cells to participate in the generation ('genesis') of the new individual (Darwin, 1868). In this regard, epididymosomes, as epididymis-derived exosomes with a wide spectrum of variation in content and size and high sensitivity to environmental stressors, could be considered as an attractive candidate for the storage of RNA content, changing the epigenome of the next generations, and allowing the reappearance of ACs of ancestors (Simon *et al.*, 2018).

Therefore, in this study, we will briefly discuss Darwin's pangenesis theory and its possible relations with epididymosomes, involving in the transfer of the required proteins and non-coding RNAs to sperm before they reach the vas deferens (Simon *et al.*, 2018).

Darwin's pangenesis theory

Mirbel and Lamarck were the first scientists who proposed the cell theory for the living organism, and after them, Rudolf Virchow, Schleiden and Schwann expanded the cell theory (Gerould, 1922; Swanson, 1960). In this regard, Virchow believed that new cells are only generated from the existing cells by self-replication, while Schleiden and Schwann discussed the generation of the new cells either by the existing cells or by spontaneous generation (Gerould, 1922; Swanson, 1960). Also, although the detailed discussion on the different cell theories is beyond the aim of this paper, it is important to note that the aforementioned cell theories could not explain IACs. Also, one may still presume that the Weismann barrier, the strict distinction between the 'immortal' germ cell lineages and 'disposable' somatic cells, is still a crucial obstacle in terms of the soma-germ cell interaction and IACs. In this regard, the word 'pangenesis' unconsciously reminds us of Jerome Cardan's statement that semen is derived from the whole body (Zirkle, 1935).

Darwin's pangenesis theory imagined particles named as 'gemmules', which are released from all ('pan') cells of the body and then, by cell–cell communication and circulation throughout the body, finally reach the germ cells, to participate in the generation ('genesis') of the new individual (Liu, 2018b). Therefore, Darwin attempted to explain the possibility of information transfer of the cells to succeeding generations by his theory and paved the way for future research on IACs. Furthermore, as this theory covers the inheritance of definite variations, it provided a complete picture of heredity alongside his biological evolution theory, which focused on the inheritance of indefinite variations, hereditary changes that randomly occur and are not induced by the environment (Darwin, 1859).

According to the pangenesis theory, Darwin supposed that gemmules are 'inconceivably minute and numerous as the stars in heaven' and 'many thousand gemmules must be thrown off from the various parts of the body at each stage of development' (Darwin, 1875). Correspondingly, gemmules were thought to self-replicate, stay in a dormant state, fuse to other gemmules or cells, be altered by environmental stressors and/or be transferred from parents to offspring after these gemmules interacted with germ cells (Darwin, 1868; Liu and Chen, 2018; Liu, 2018b). Given the inadequate evidence on Darwin's gemmules, this novel concept has been neglected or refuted for many years by scientists, including Weismann, as nobody knew the existence of these particles and their possible mode of actions (Liu and Chen, 2018).

Nucleic acids and Darwin's pangenesis

After the introduction of DNA as a substance of inheritance by Avery *et al.* (1944), Mandel and Metais (1948) indicated the existence of circulating cell-free nucleic acids in humans. As a result, they found that 90% of the circulating nucleic acid is RNA (Mandel and Metais, 1948). After Mandel's interesting finding, important questions arose regarding the possible roles of the circulating cell-free nucleic acids. Subsequently, Sopikov (1954) reported evidence on the heritable alterations by blood transfusion and then hypothesized that the circulatory cell-free DNA is probably responsible for such a heritable induction. In the later years, several scholars reported the existence of circulatory DNA in patients with cancer (Bendich, 1961; Bendich *et al.*, 1965; Liu, 2018c). Consequently, they emphasized that DNA could be released by tumours and, through the bloodstream, could reach target cells and be taken up by them, as circulatory DNA has been considered as a 'liquid biopsy' nowadays for patients with metastatic cancer (Bendich, 1961; Bendich *et al.*, 1965; Wan *et al.*, 2017). A growing body of evidence indicates that Darwin's gemmules are linked to cell-free nucleic acids and extracellular vesicles due to the similarities in their release and movements compared to gemmules, and the fact that they consist of nucleic acids, the substance of inheritance (Barry, 2013; Aucamp *et al.*, 2016; Chen *et al.*, 2016; Liu, 2018c; Ho, 2019; Bonduriansky and Day, 2020).

It has been indicated that RNA may be the first genetic molecule due to its enzymatic activity which catalyses its self-replication (Crick, 1968). When it comes to the relationship between RNA and IACs, the transformation of the goldfish's tail from double to single after injection of carp's ovarian eggs-derived mRNA to goldfish's eggs has been reported (Tung and Niu, 1973). Moreover, similar results have been obtained by injecting different kinds of coding and non-coding RNAs into eggs to generate specific ACs. Accordingly, RNA becomes one of the important aspects of IACs (Liu, 2018a,c; Bohacek and

Rassoulzadegan, 2020). A growing body of evidence has also indicated that a wide spectrum of effectors, such as food (Love, 1909; Newberne and Young, 1973; Johnson, 1990; Ng *et al.*, 2010), acquired habit (Pawlow, 1923; McDougall, 1927, 1930; Lindqvist *et al.*, 2007; Nätt *et al.*, 2009), immunity (Guttmann and Aust, 1963; Lemke *et al.*, 2004; Steele, 2009), light (Durken, 1923), chemicals (Spergel *et al.*, 1971, 1975; Sano, 2002; Akimoto *et al.*, 2007) and age effects, (Redfield, 1917; Dufton, 1932; Jablonka and Lamb, 1999; Liu *et al.*, 2011), could be inherited by the offspring. The exact mode of inheritance has not yet been completely unravelled. Nevertheless, many crucial questions have been raised regarding the mechanism of action of RNA in the transfer of environmentally-induced changes to offspring. At this point, Steele (Steele, 1981) reported that changes in somatic cells could be transferred to the germ cells in the form of RNA, which is then transcribed to DNA by the reverse transcriptase and then combined into the germline DNA. He named his hypothesis as 'somatic selection' (Steele, 1981). Accordingly, Spadafora (2018) also recently declared that the transcriptase activity of retrotransposons called LINE-1 in the sperm head could be responsible for the non-mendelian transfer of parental genetic information to the next generation (Spadafora, 2018). Interestingly, it has been identified that the majority of the circulating DNA consists of transposable and/or repetitive elements, including LINE-1 (Bronkhorst *et al.*, 2018; Grabuschnig *et al.*, 2020). This further supports the connection between pangenesis gemmules and circulating nucleic acids. Accordingly, Steele *et al.* (1998) in his book entitled 'Lamarck's Signature: How Retrogenes Are Changing Darwin's Natural Selection Paradigm' considered his 'somatic selection' theory as a modern insight into Darwin's pangenesis (Steele *et al.*, 1998).

The roles of various types of non-coding RNAs such as miRNAs and t-RNA fragments (tRFs) have been reported in sperm biology as well as in transgenerational inheritance (Bohacek and Rassoulzadegan, 2020). The regulatory functions of these RNAs vary in sperm, testis and epididymis and, interestingly, such differences have been observed in the diverse compartments of the epididymis, such as caput, corpus and cauda (Nixon *et al.*, 2019). Moreover, it was shown that environmental effectors can change the composition of non-coding RNAs in these compartments, depending on the exposure timing and developmental stage, as and can impact sperm RNA composition and deliver certain different non-coding RNAs to the embryo during the fertilization process (Bohacek and Rassoulzadegan, 2020). In this regard, Sharma *et al.* (2016) reported an interesting study performed on the roles of tRFs during sperm maturation. They examined the effects of a parental low protein diet on the composition of sperm non-coding RNAs and on metabolic changes in the subsequent generations (Sharma *et al.*, 2016). Finally, they indicated that epididymis-derived exosomes, i.e. epididymosomes, are responsible for the transfer of tRFs to sperm during its journey from the testis to fertilization (Sharma *et al.*, 2016).

Epididymosomes

Epididymosomes, as epididymis-derived exosomes, have a wide spectrum of variation in their content and size, depending on their secretion pattern from the different compartments of epididymis (Rejraji *et al.*, 2006). Interestingly, in contrast with the sperm membrane, which tends more to be fluid, epididymosomes have rigid membranes, and this rigidity increases from the caput to cauda (Rejraji *et al.*, 2006). Consequently, as expected, epididymosomes play important roles in sperm maturation, sperm motility and their ability to penetrate the oocyte during the fertilization process (Simon *et al.*, 2018). The transfer of the required proteins from epididymosomes to sperm has been regarded as the main process in sperm maturation before the sperm reach the vas deferens (Simon *et al.*, 2018). Furthermore, epididymosomes can distinguish between viable and dead sperm and can also protect the viable sperm from oxidative stress stemming from dead sperm (D'Amours *et al.*, 2012). Notably, all these vital functions, acquired by sperm during the maturation process, at least partially depend on the transportation of proteins from epididymosomes to them (Simon *et al.*, 2018).

As mentioned in the previous section, non-coding RNAs, and especially tRFs, are involved in the inheritance of ACs in the succeeding generation (Bohacek and Rassoulzadegan, 2020). Moreover, it seems that epididymosomes are very sensitive to environmental stressors, which are probably responsible for translating external information to the germ cells (Bohacek and Rassoulzadegan, 2020). However, some important questions such as the exact roles of each non-coding RNA classes remain unanswered; for example, we still do not know which type of RNA can transfer specific environmental information to the germ cells, total RNA, miRNAs or tRFs? Injection of different types of RNAs has sometimes brought conflicting results (Bohacek and Rassoulzadegan, 2020), thus more studies are needed to clarify the exact roles of the epididymosomes-derived non-coding RNAs in IACs.

Epididymosomes: the black box of Darwin's pangenesis?

Since Darwin's pangenesis was presented to scientific societies, it has been faced many ups and downs. The theory of the transfer of the environmental information to the next generations was too far-fetched due to inadequate evidence as well as contrary theories such as the germ-plasm theory (Aucamp *et al.*, 2016; Liu and Chen, 2018; Liu, 2018b). If we consider the progression of Darwin's pangenesis by four main points (environment, somatic induction, accumulation of the information in the germ cells and inheritance by the offspring), the knot in this theory would be the storage of genetic and epigenetic information in the germ cells by the immediate or distant ancestors. In this regard, Darwin, in his theory, discussed the long-dormant ancestral gemmules and atavism (i.e. the reappearance of some ACs similar to that of their ancestors that were not presented by their immediate parents) (Liu, 2018b). The key point of atavism is probably the power of genetic and/or epigenetic dormancy, which in turn could lead to the loss of some ACs in the immediate parents and sudden reappearance of them in the next generations (Liu, 2018b). Although this part of the pangenesis theory has been neglected for many years, the storage ability of germ cells may be hidden under the word of 'dormancy'. If this is possible in any way, what is its underlying mechanism? These questions reflect some of the scientific gaps regarding the 'dormancy' in the 'pangenesis theory'.

Moreover, it has been shown that the circulatory exosomes, which can contain over 90% of circulating DNA (Fernando *et al.*, 2017) are affected by environmental stressors (Spadafora, 2018). These altered somatic nanovesicles could pass the Weismann barrier and reach the paternal germ cells (Fig. 1) (Spadafora, 2018). Afterward, the RNA content of exosomes is taken up by the sperm head and either is reversely transcribed to cDNAs using LINE-1 retrotransposones or is stored as RNA packages (Fig. 1) (Spadafora, 2018). The final destination of cDNAs is their storage in sperm or possibly their delivery to oocytes during the fertilization process. In this regard, the main question is that, when circulatory exosomes pass the Weismann barrier, where is the content of these vesicles discharged and stored? If we want to go again through Darwin's pangenesis, we can state that, interestingly, Darwin also discussed the possible interactions of 'gemmules' with each other and also the interactions of other gemmules with various patterns of affinity (Liu, 2018b,c). Therefore, in the mirror of his theory, circulatory exosomes could interact with epididymosomes and discharge their contents. Subsequently, the protein and RNA cargo would be transferred by epididymosomes to sperm during sperm maturation and then delivered to the embryo during fertilization, followed by inheritance by offspring (Fig. 1) (Bohacek and Rassoulzadegan, 2020). However, when it comes to 'dormancy' and 'atavism', we believed that epididymosomes could be considered as an attractive candidate for the storage of RNA contents, changing the epigenome of the next generations, and allowing the reappearance ACs of the ancestors. Therefore, epididymosomes, as the black box of Darwin's Pangenesis, can unravel parental life history and also disclose the historical events, which affect parental life.

Conclusion

In the current review, we discussed the importance of the environmentally-induced information being transferred from parental germ cells to offspring and then briefly explained some of the related evolutionary biology theories with their pros and cons. In this regard,

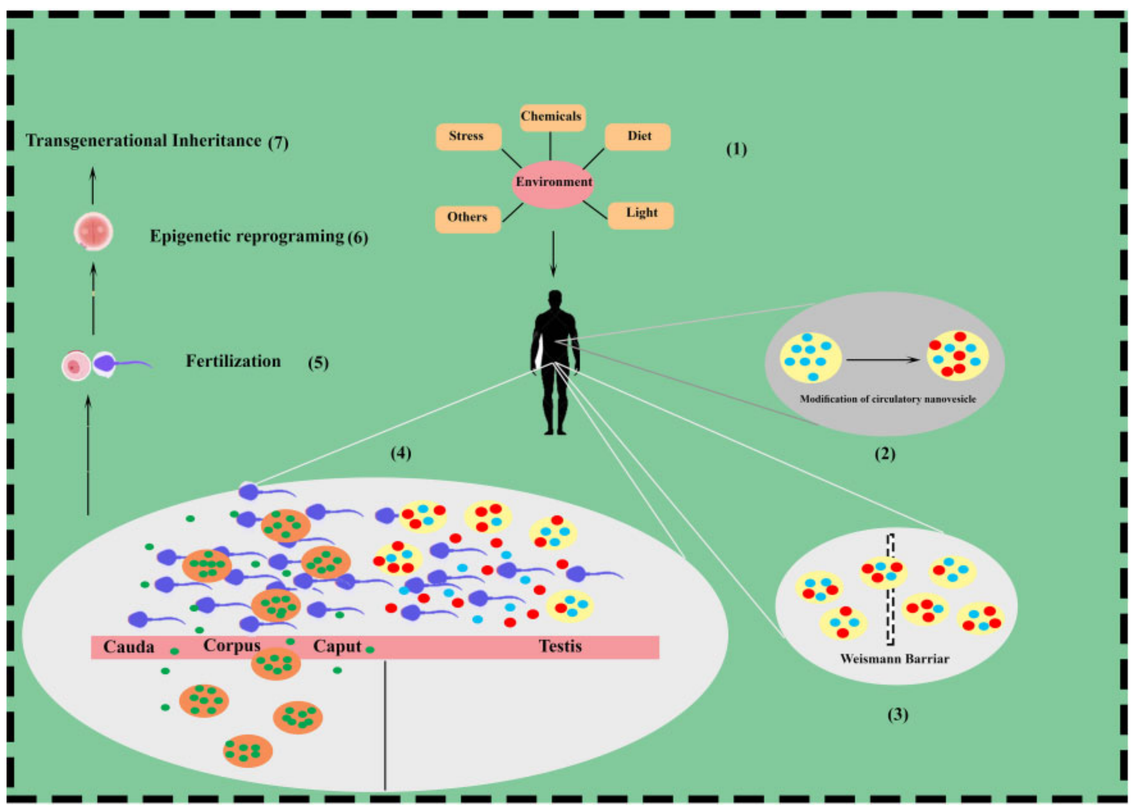

Figure 1. The effects of environmental factors on transgenerational inheritance based on Darwin's pangenesis theory. (**1**) Different types of environmental factors such as stress and diet could affect parental nanovesicles during a man's life, producing (**2**) modified circulatory nanovesicles, which (**3**) may pass the Weismann barrier, and enter the germ cells. (**4**) After entering the germ cells, in the testis, sperm may take up the contents of the modified circulatory nanovesicles especially the non-coding RNAs. The rest of the modified circulatory nanovesicles could be reserved in the epididymis, and/or communicate with other nanovesicles like epididymosomes and share the molecular contents. (**4**) When sperm pass along the epididymis during the maturation process, epididymosomes release and could transfer essential RNA packages for better sperm maturation and fertilization, and possibly development of the future generations. These packages may be maintained in the epididymis, as the epididymosomes. (**5**) Once sperm have obtained the required contents from the epididymosomes, one could transfer these non-coding RNAs to the oocyte at the fertilization process, and (**6**) in turn cause changes the epigenome of the embryo. (**7**) Finally, the initial effects of the environment lead to significant changes, occurring during sperm maturation and fertilization and could be transferred as certain cocktails of non-coding RNAs to future generations. **Yellow Circles**: circulatory nanovesicles; **Orange Circles:** epididymosomes; **Blue Circles:** unmodified non-coding RNAs; **Red Circles:** modified non-coding RNAs; **Green Circles:** modified epididymosomes-derived non-coding RNAs.

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we especially found Darwin's pangenesis as a modern theory in cellular and molecular biology. Moreover, we believe that the translation of Darwin's views in 'pangenesis theory' to modern biology might open some new windows for designing novel studies for evaluating the transfer of environmental effects, affected by parental germ cells, to the next generations. With a specific focus on epididymosomes, we need to know the exacts effects of the various types of environmental stressors with different doses and/or various timing of exposure to the composition of different classes of non-coding RNAs at different developmental stages or germ cells differentiation processes. This must be performed because we indicated that the effects of certain environmental influence on RNA content of different parts of epididymis such as caput and cauda were very diverse. Moreover, scholars have interestingly shown that cauda- and caput-derived sperm had divergent results during the fertilization process as well as embryo development. Furthermore, we should conduct well-designed studies for tracking the destination of exosomes undergoing the influences of particular environmental stressors until their appearance in the germ cells and possibly in the next generations. Accordingly, a tracking system could help in understanding the direction of certain exosomes in the circulation and also in the epididymis and germ cells, which may pave the way to unravel the underlying mechanisms in IACs. Therefore, on the one hand, the high sensitivity of epididymosomes to the environmental effectors and their action as the black box for evolutionary biology gives us hope, but on the other hand, studying such variable nanovesicles will take a long time and a thorough understanding of the potential mode of actions will be difficult.

Finally, we would like to emphasize the importance of the basic evolutionary theories, in which their whole details have not thoroughly been investigated up to now. In this regard, Fisher and Stock (1915) in their book in 1915 have written about Professor Freeman, who warned his students 'mastery of one great book was worth any amount of knowledge of many lesser ones' and also said that 'How many biological students of today have read The Origin?'. Altogether, the known functions of epididymosomes are very similar to Darwin's gemmules, and his seemingly simple theory may have been very attractive with astonishing details which remain to be clarified.

Data availability

All the data underlying the arguments put forward in this review are referenced within the paper.

Authors' roles

H.R.N.: conception and design, drafting of the article, critical revision for important intellectual content. V.S.: drafting of the article. Y.F.: drafting of the article. N.F.-M: literature review and acquisition of data. Z.B.-A.: critical revision for important intellectual content. S.N.: software and visualization. M.N.: critical revision for important intellectual content and final approval of the version to be published.

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Conflict of interest

The authors declare that they have no conflict of interest.

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