Old Herborn University Seminar Monograph 32: Ageing and the Microbiome.
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AGEING AND THE MICROBIOME: SUMMARY OF THE 32ND OLD HERBORN UNIVERSITY SEMINAR AND THE DISCUSSIONS

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Following a warm introduction, *Volker Rusch* opened the Seminar. The focus of this year's meeting was ageing, with an emphasis on whether the gut microbiome played any role in the process.

Michael Zasloff presented a brief introductory lecture ("*Some Insights about Ageing from a Shark and an Oyster*"). He pointed out that the two documented longest-lived animals were the "Ming Clam" (400 years) and the Greenland shark (500 years). Their survival obviously required non-senescent stem cells, and an immune system that could protect against all potential pathogens. He pointed out that the details of the animal "ageing clock" is unknown. Zasloff finished his presentation with a discussion of recently published studies in mice that suggested that hypothalamic inflammation leading to the reduced expression of certain hypothalamic hormones (i.e. GnRH) drives peripheral ageing. These observations suggest that an "elixir of youth" might be more real than we believe.

Robert Pignolo presented a general overview of the physiology of human ageing from the perspective of a research gerontologist ("*Physiology of Human Ageing*"). He made the point that it becomes difficult to separate ageing per se from the onset of disease processes that are associated with ageing. If we could slow down ageing, how would we document the response? One approach is to focus on the loss of function of a system, such as the GI tract, the immune system, the endocrine system and the nervous system cataloguing the known progressive changes that occur in the otherwise healthy person. He highlighted the known progressive changes that occur with respect to specific tissues, such as muscle, bone, skin and adipose tissue. He stressed that a deeper analysis of these processes could be extended mechanistically to dissect the specific pathways leading to the age-related changes. Ageing in humans is frequently associated with a loss of resilience and those factors that increase an individual's fragility, and reduce their probability of recovery. He stressed that much current interest in human ageing is focused on the mechanistic basis of reduced stem cell survival, cellular senescence and the underlying mechanisms responsible for chronic inflammation. In particular, considerable research in human ageing is now focused on drugs that deplete senescent cells.

Brian Kennedy posed the provocative question of whether we could intervene to delay human ageing ("*The Age of Ageing: Can We Intervene*"). He reviewed the known conditions that have been shown in various systems to extend lifespan, such as caloric restriction. Of particular interest were small molecules that had already been developed and were currently in human use for various medical conditions. In particular he highlighted metformin and rapamycin. While the mechanism

underlying the benefit of metformin was still unclear, rapamycin's effects on ageing appear to be attributable to mTOR inhibition. mTOR regulates protein synthesis in response to nutrient and energy availability. Recent studies suggest that mTOR inhibition extends stem cell survival. He presented remarkable studies that supported the effect of mTOR inhibition on age related stem cell loss in the murine trachea.

He then tackled the persistent theme of how best to measure the impact of an intervention that delayed ageing in humans. One promising potential biomarker is the accumulation of methylation across the 450K CpG site assessable in the human genome. As an individual age the number of methylated CpG sites increases providing an "epigenetic clock" that might be of use. Kennedy described another approach his colleagues have explored, that being facial recognition. Facial recognition technology has advanced to such an extent that chronological age can be reliably estimated through this modality, possibly providing a tool to assess the impact of anti-ageing interventions.

Dario Riccardo Valenzano presented his work with the turquoise killifish as a new model animal to study the ageing process ("*Life Span Regulation by the Gut Microbiota in the Naturally Short Lived African Turquoise Killifish*"). The killifish has a lifespan of between11-26 weeks, bridging the brief lifespan of *C. elegans* (3 weeks) and *Drosophila* (9-13 weeks) and the longer lifespan of the mouse (130 weeks). This species is indigenous to Africa. The genomic sequence was published in 2015 as well as techniques to genetically modify the animals. Ageing in killifish is characterized by dramatic skeletal disturbances including a form of scoliosis, reduced capacity to learn, decreased fertility, slower overall movement, sarcopenia, and gut fibrosis. Valenzano noted that the microbiome of the killifish changes dramatically as the animal ages, and then posed the question of whether the gut microbiome influenced ageing. The most abundant taxa in its microbiome are shared with mammals, and common microbial taxa are shared between animals caught in the wild and those maintained in captivity. Can the course of ageing be changed by altering the microbiome? Microbial transplants from young or old fish were introduced into middle aged fish that had been pretreated with antibiotics. The most dramatic outcome of this study was with young microbial transplants: survival was extended, swimming activity was increased to a level normal for young animals, changes were seen at the transcriptional level in the intestinal tissues. He hypothesized that the effects observed could in part be due to the differences in serum metabolites associated with the young or old gut microbial communities and the interaction with host physiology.

Christoph Kaleta presented a broad overview of ageing ("*Evaluating Potential Contributions of the Microbiome to Ageing Pathologies*"). His overriding thesis was that a major factor in the ageing process is due to the accumulation of dead or damaged cells, which, in turn, provokes chronic inflammation. Might the ageing microbiome impact on the clearance of senescent cells? Perhaps metabolites of microbiome origin play a role. And if so, we might be able to intervene.

Thomas C. G. Bosch presented an expansive perspective on ageing focusing on his studies of *Hydra* ("*Ageing and the Microbiome: Lessons from Non-Senescent Models*"). He stressed that both genetics and environment play a role in longevity. He highlighted the remarkable role of FoxO in the biology of *Hydra*, FoxO being one of the

two genes that have been associated with longevity in humans. A three classes of stem cells in *Hydra* express FoxO, and genetic inhibition of FoxO expression slows down the population growth rate in this animal. Remarkably, FoxO also plays a role in managing the microbiome of *Hydra*. It turns out that FoxO is required for expression of antimicrobial peptides, which in turn, regulate the composition and density of the microbiome. So, by virtue of FoxO's integration into the design of *Hydra*, it controls cross-talk between the stem cell and the microbiome. This relationship is dramatically altered in the setting of a *Hydra* strain in which a tumour has arisen. The presence of the tumour, which is of an epithelial nature, dramatically alters the microbiome, increasing the proportion of spirochetes. These spirochetes are not passive bystanders, since their elimination with antibiotics causes regression of the tumour. Re-inoculation with these bacteria causes a recurrence of the tumour. Careful analysis of the microbial interactions involved, demonstrate that a *Pseudomonas* strain is also required for tumorigenesis, implicating the two species in this process. Bosch stressed that this story underscores the principle that the physiology of an organism cannot be understood without including the microbial elements with which coexists, without viewing the entirety of the holobiont.

Wolfgang Kunze focused on the ageing of the enteric nervous system (ENS) and how gut microbes might influence the activity of the ENS ("*Ageing and Gut to Brain Signalling*"). He reminded us that gut microbiota change with ageing and that feeding *Bifidobacteria* to mice has been shown to increase lifespan. He suggested that gut microbes could contribute to ageing through neural communication to the brain, for example, via the vagus. When the *Lactobacillus* strain, JB-1 is introduced into the mouse gut, 90% of the signals stimulated by JB-1 are transmitted through vagal afferents. He highlighted that as we age the ENS progressively deteriorates. Peristalsis is reduced. Afferent activity decreases. Ion channel activity, such as the Ik potassium channel, diminishes. The consequences of this deterioration can be observed in the brain. For one, stimulation of the vagus has been shown to activate growth hormone releasing factor in the hypothalamus, demonstrating a clear neural linkage between the hypothalamus and the gut. Introduction of JB-1 or squalamine into the intestine (studied *ex vivo*) leads to increased vagal firing in aged mice, suggesting that the diminished activity of the aged ENS can be corrected therapeutically.

Josef Anrather introduced the concept that inflammation within the brain surrounding stroke could be modulated by the gut microbiome ("*From the Gut to the Brain: The Roles of Microbiota in Stroke*"). He described several models of stroke as studied in rodents. The basic premise of his hypothesis is that IL-17 T cells are major players in the inflammatory events that follow a stroke, that the gut is the major reservoir of IL-17 T cells, and that the microbiome plays a role in the differentiation of IL-17 T cell population. Following a stroke intestinal IL-17 T cells traffic to sites such as the meninges, and then into the brain. By altering the population of intestinal microbes, it is possible to influence the dendritic cells of the intestinal lamina propria to shift the T cell population towards Tregs. He has begun studies that suggest that a factor extractable from the "good" flora can recapitulate the beneficial effects on the intestinal T cell population of the gut. These studies suggest an entirely novel understanding of the pathophysiology of the events that follow a

stroke, and treatments that might be developed.

Janelle C. Arthur focused on the role of the microbiome in colorectal cancer, a disease of ageing ("*The Microbiome and Cancer*"). She reminded us that the current explanation for the occurrence of colorectal cancer is chronic inflammation, caused either by microbes or by organic compounds of dietary origin present in faeces. From studies with IL-10 knock out mice, an animal that develops colorectal cancer, it is clear that the microbiome plays a role, since germ-free animals of this strain do not exhibit this phenotype. Arthur asked whether specific microbes might be involved. Since inflammation precedes the appearance of cancer in these animals, might inflammation produce a change in the microbiome that encourages the appearance of carcinogenic microbes? To answer these questions, she used germ-free IL-10 knock out mice. The animals were inoculated with SPF microbiota, then treated with azoxymethane, a chemical that provokes both intestinal inflammation and colorectal cancer. The microbiome was then examined and compared with the initial inoculum with respect to its species. Treatment with Azoxymethane led to a 100-fold increase in *E. coli*. If the same experiment was performed with either a pure culture inoculum of *E. coli* or *E. faecalis* (rather than the SPF inoculum) only the *E. coli* inoculated mice developed significant colorectal cancers. It appears that strains of *E. coli* that harbour the polyketide synthase (*pks)* pathogenicity island appear to be the culprits. A specific substance produced by these strains of *E. coli* can induce cancer independently of inflammation. The product of the *pks* gene cluster is a poorly characterized molecule called Colibactin which has been shown to cause chromosomal damage by an unknown mechanism and requires contact between the bacterial cell and the epithelium. These studies suggest that age related changes in the microbiome could be a cause of colorectal cancer, and clearly amenable to intervention.

The formal Academic ceremony followed the completion of the Seminar, with the granting of Honorary Professorships at the Old Herborn University to the speakers.