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Editorial Note

Tumour travel tours – Why circulating cancer cells value company

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ABSTRACT

Welcome to the New Year and a new issue of the Biomedical Journal, where we learn that travelling with company boosts the metastatic potential of circulating tumour cells, as well as that a worm could be an excellent model to study antidiabetic drugs. In addition, we discover another pair of molecular scissors for genetic engineering, how exactly *Leptospira* wreaks havoc on its run through the host organism, and that hyperparathyroidism brings its own risks, but does not worsen the outcome of papillary thyroid carcinoma. Furthermore, the importance of taking into account differing beauty ideals for aesthetic surgery surveys is discussed, alongside the question how bad isolated local recurrence is in the case of HR + breast cancer. Finally, we find out that virtual colonoscopy deserves more credit, that the first medical experiment in space was all about the H-reflex, and that it is possible to survive advanced necrotising fasciitis of the face and neck.

Spotlight on reviews

Tumour travel tours – why circulating cancer cells value company

Everybody needs a friend, claimed the iconic landscape painter Bob Ross in one of his tutorials. Ross was speaking of trees, but everyone who has to deal with cell culture knows perfectly well that cells too need company, either in the form of dense seeding, feeder cells, or molecular tricks like adding Rock inhibitor to the medium [1]. Especially stem cells are particularly sensitive to loneliness, which makes them either die or differentiate [2]. Circulating tumour cells (CTCs), the offspring of the primary tumour that set out into the bloodstream and

lymphatic system to instigate metastases, may be the ultimate villains, but no exception to the rule, as Nicola Aceto explains in his review [3].

By now, it is well accepted that the tumour microenvironment and host immune system can be serious accomplices during primary cancer growth [4]. Twelve years ago, it was shown that the simple co-injection of matrigel potentiated the tumorigenic abilities of a large fraction of melanoma cells, thus triggering the contestation of the cancer stem cell supremacy [5]. To date, the tumour niche is intensely studied, as it became clear that this complex mixture of endothelial cells, fibroblasts and immune cells contributes greatly to many cancer hallmarks: angiogenesis, chronic inflammation, stem cell-like properties and growth signalling [6]. Unsurprisingly, this got it also straight into the therapeutic firing line [7].

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Cancer and the immune system, for their part, have an even more complicated relationship. On the one hand, cancer immunotherapy carries currently the highest hopes on its shoulders, with sophisticated methods such as chimeric antigen receptor T cells, or specific antibody therapies [8]. On the other hand, most solid tumours are extremely talented at subverting members of the immune system, including macrophages, neutrophils, and T cells [9]. Macrophage infiltration for example foreshadows bad prognosis, as it stimulates angiogenesis, tumour cell survival and extravasation [10].

What exactly happens once a cancer cell decides to leave the nest however remains more elusive, as CTCs are sparse, mobile, and hidden amidst the bloodstream. The fact that CTCs can bring company however has been theorised a long time ago, but experimentally proven only recently to be shed by many different cancers. Aceto explains the technological innovations that made the latter possible, and outlines the two main strategies in use, based either on antigenic staining or size selection by microfluidics. Subsequently, he outlines the biological features of homotypic clusters, composed of cancer cells only, and heterotypic clusters, a mix of cancer

with stromal or immune cells. A key feature of homotypic CTC clusters is the presence of intercellular junctions, a feature that rings a bell, once again, for embryonic stem cell aficionados. It is no secret that tumour cells frequently hijack pluripotency pathways for their advantages, such as unlimited proliferation, immortality and sturdy features [11]. As the author accurately points out, cell–cell junctions are required for the expression of the core set of pluripotency transcription factors, both in native stem cells and cancer cells. Heterotypic CTC clusters consist basically in cancer cells bringing along a little piece of their original stromal niche: fibroblasts, endothelial cells or immune cells. The familiar feeling seems to have the same comforting effect as the primary tumour microenvironment, including better survival and boosted proliferation.

Altogether, travel company, be it by other cancer or stromal cells, appears to have the same downstream effect on CTCs: faster engraftment due to earlier entrapment in small blood vessels, increased fitness and dedifferentiated features, thus leading to more frequent and aggressive metastases (Fig. 1).

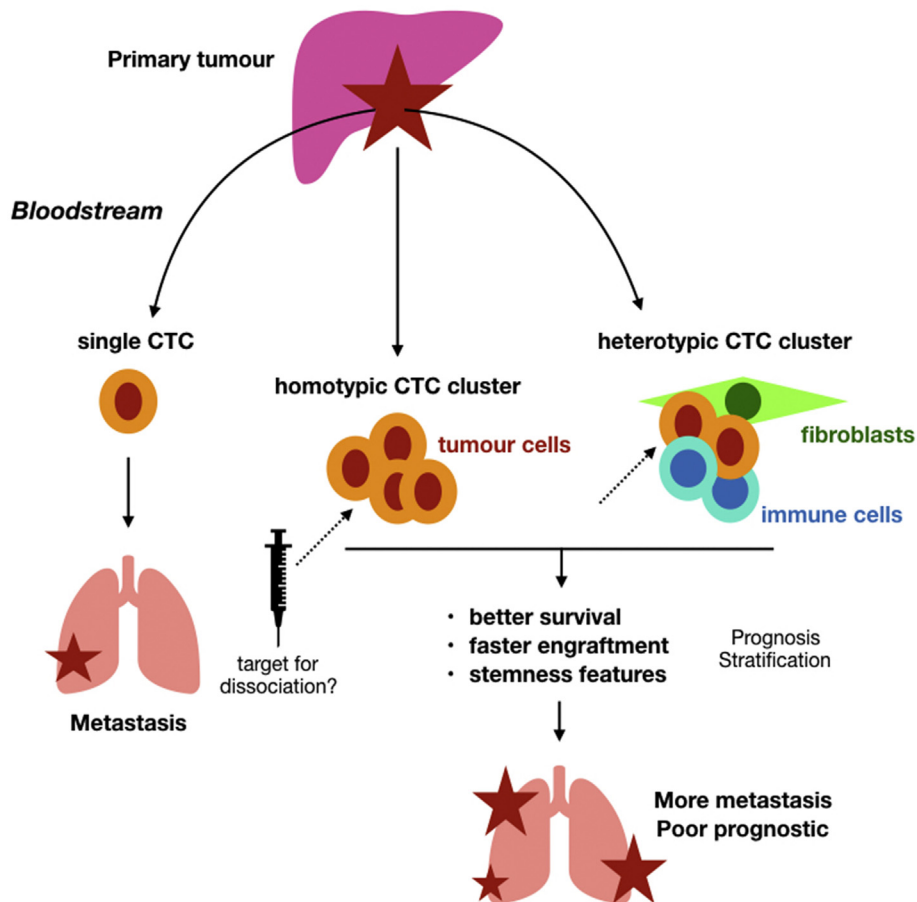


Fig. 1 Cluster formation potentiates the metastatic potential of circulating tumour cells. Circulating tumour cells (CTCs) are cells that have migrated from the primary tumour into the bloodstream or the lymphatic system and can cause metastasis in distant sites. Clustering with other tumour cells (homotypic), or cells from the cancer microenvironment (heterotypic), improves CTC fitness and fastens engraftment, thus increasing their metastatic potential. CTC cluster detection could serve for better patient stratification or to gain information on the tumour, alternatively targeting CTC clusters for dissociation could lower the metastatic risk.

Now, seen in this way, this is rather depressing news, at least for the host organism. Aceto attempts nonetheless to slightly lighten the mood by stressing the diagnostic and eventual therapeutic value of the CTC cluster discovery. Diagnostic-wise, they could fine-tune patient stratification and offer valuable clues on tumour composition and genetics. Regarding therapeutics, targeting these clusters for dissociation would be tempting, despite that the technicalities are yet undetermined.

At any rate, CTCs on the road and eventual travel companions need to be further studied, if we hope to ever develop an efficient roadblock for them before they settle down.

Spotlight on original articles

Send in the worms: using C. elegans in order to understand anti-diabetic drugs

Sydney Brenner, one of the key figures of the rise of molecular biology during the 20th century - notably for the discovery of messenger RNA - received the Noble prize in Physiology or Medicine in 2002. His Nobel lecture *Natures Gift to Science* is dedicated to “the fourth winner of the Nobel prize this year” [12], and referred to no one else than *Caenorhabditis elegans*. In 1974, he elected the 1-mm-long roundworm as his model system of choice in order to study “the complex structures found in higher organisms”, notably the development of the nervous system [13]. The tiny worm had the right size for electron microscopy, lived happily in a dish filled with *E. coli*, self-fertilized and produced about 300 new nematodes every 3.5 days [12]. Brenner's first study reported hundreds of mutants and related genes [13]. Since then, *C. elegans* has become an evergreen model organism for a broad scientific community, and despite the latest trend to go as human-like as possible for some studies (like miniature brains in a dish [14]), the worm has never lost its pertinence for the understanding of both normal ontogenesis and various human pathologies, including neurodegenerative diseases [15].

Hsiung et al. add another proof for the statement in their study published in this issue of the Biomedical Journal, by demonstrating that the nematode can also be used to as a model for human metabolic failings [16].

Metabolic defects are keystones to countless pathologies, and their incidence is on the rise, to a degree that many publications nowadays refer for instance to an ongoing “diabetes epidemic” [17]. Only recently though, the central role of mitochondria in disorders like diabetes and obesity has received the deserved attention, and been included as a potential therapeutic target [18]. One target might be CISD-1, termed mitoNEET until not too long ago. It belongs to a recently discovered family of iron-sulphur proteins (CISD1-3), which are all linked to the mitochondria in humans, and implicated in a large array of functions ranging from respiration and iron homeostasis to protein biosynthesis [19]. CISD-1 in particular was detected in 2003, when Colca et al. tried to decipher the mechanism underlying the beneficial action of thiazolidinedione against type 2 diabetes, known since the beginning of the 1980s. It turned out that the drug, thought to mainly activate the nuclear receptor PPAR γ , also cross-linked

a novel mitochondrial protein [20]. Nevertheless, it is still unclear how exactly thiazolidinedione and similar compounds impact on CISD-1, and what the downstream molecular events relevant for diabetes treatment are.

This lingering question prompted Hsiung and colleagues to look for a suitable *in vivo* model system that would combine ease of study with conservation in humans: it was time to call in the worms.

Not only is the fate of every single cell of *C. elegans* across development well studied and documented by now [21], the scientific worm community has also created valuable databases for any nematode-related -omics, first and foremost the WormBase website.¹ Similar good annotation practices certainly facilitated the hunt for CISD equivalents, and indeed, the authors successfully uncovered a homolog for the human *Cisd-1*, further referred to as *cisd-1*, and two homologs for CISD-3. Expression of a 143 amino acid protein corresponding to CISD-1 is further confirmed experimentally. Like the human version, the *C. elegans* CISD-1 is subsequently shown by complementary techniques to be located in the outer mitochondrial membrane.

In order to investigate its function, a CISD-1 mutant is generated by deleting a major part of the gene. Surprisingly, both motility and lifespan of the mutant worms does not differ from the wild-type, however, the authors make an educated guess - based on location and known function of the homologous proteins - and decide to investigate thoroughly both morphology and performance of the mitochondria in the mutant worms.

Shape and function are known to be closely related for this special organelle, once an organism on its own, after all [22]. “Function” alone encompasses energy production, cell signalling, calcium homeostasis, stress regulation and cell death management. In addition, over the recent years, it has become clear that mitochondrial shape is a major regulatory factor for the cellular bioenergetics capacity [23]. In other words, being in a bad shape has to be taken literally when it comes to this cellular compartment [24].

As suspected, the CISD-1 mutant mitochondria display a markedly altered appearance, in the form of a hyperfused morphology. This kind of phenotype (in this case due to a DRP1 mutation) has been shown for instance to play a role in severe axonal neuropathy [25]. Surprisingly though, hyperfused mitochondria have also been correlated with longevity, alongside elevated autophagy activity [26].

Confirming the shape-function connection, the authors detect elevated reactive oxygen species (ROS) and superoxide production by the mutant mitochondria, paralleled by a decrease by half of ATP production, and lower glucose levels.

Lastly, Hsiung et al. show that several anti-diabetic drugs have effects similar to the CISD-1 mutation on wild-type worms, including mitochondria hyperfusion, as well as a decrease of ATP and glucose levels. These results in turn suggest that CISD-1 is as well the target of these compounds in *C. elegans*.

In a nutshell, all results boil down to the encouraging conclusion that the nematode would be a fitting model to study the CISD-1 and drugs targeting the latter in a setting mimicking human diabetes.

¹ wormbase.org.

Also in this issue

Reviews

The curious Cas(e) of CRISPR-Cas12a: an alternative pair of molecular scissors

No doubt that the CRISPR-Cas endonucleases, the molecular tools that allows for cutting double-stranded DNA at an exact location, are by far the most publicised biomolecular innovation of the recent years, both among the scientific community and the general public. Sparking hopes and fears worthy of science fiction novels, the field has already experienced its share of drama, with the ongoing fight over the patent [27,28], and scandals, with the generation of the “CRISPR babies” [29,30]. Nevertheless, apart from having become the new standard for generating experimental knock-out models, solid proof has been generated that the method holds the potential to correct serious genetic defaults, such as sickle cell anaemia [31]. Moreover, “prime editing”, the *in situ* directed modification of single nucleotides without the requirement of the cellular DNA repair machinery, hit the headlines recently [32].

Most people immediately equate CRISPR Cas with the Cas9a enzyme, which is indeed the most broadly used one, yet the original inventors – bacteria and archaea – possess two classes and six types of CRISPR-cas systems, and some of these might also be suitable to be repurposed into “molecular scissors”. Paul and Montoya dedicate their review to the class II type V CRISPR-Cas12a system, an alternative, so far underappreciated genome-editing tool, which has already proven its worth by correcting muscular dystrophy in human cells and mice [33]. The authors first explain thoroughly how the endonucleases carry out their native function in prokaryotes, followed by a detailed account on Cas12a structure and operation mode. Subsequently, by comparing the enzyme with Cas9a, they highlight its advantages, such as the requirement of only one RNA molecule and the possibility to perform multiplex gene regulation, and drawbacks, notably its indiscriminate single strand DNA cleavage activity [34]. Given that the prime editing complex is so far extremely hard to get into cells due to its size, Cas12a has solid chances to encounter broad applications both in genome editing and bioengineering.

Fast and furious: Leptospira quickly invades the entire host and causes massive inflammation

Zoonoses, originating from animal to human transmission, make up for 60% of all infectious diseases – the currently circulating Wuhan coronavirus is just the latest illustration. Eradication of the pathogen is almost impossible, as it resides in a huge reservoir and often does not cause severe symptoms in the animal host [35]. However, the media-fuelled distortion of risk assessment regarding the newest recruit to the set obscures the way deadlier classics of the zoonosis collection. Sun and colleagues set the number straight by reminding us that leptospirosis, a systemic inflammatory disease caused by *Leptospira* bacteria, claims ten thousand victims per year and is expanding its operating range [36].

What makes *Leptospira* a formidable enemy is the fact that the bacterium is permanently on the move: it quickly transgresses mucosal and skin barriers, pierces through blood

vessels and renal tubule epithelia, spreads in no time to many organs, as well as the cerebrospinal fluid, and hijacks the host cell endo- and exocytosis machineries. On the run, *Leptospira* leaves behind a trail of destruction, both through its ability to induce apoptosis in multiple cell types, and through a massive provocation of the host immune system, turning it into its own enemy by excessive inflammation. Haemorrhage and multiple organ failure are the worst-case outcome of the infection, with 50% mortality at this stage [37].

Here, Sun et al. focus on the recent progress made in understanding *Leptospira*'s virulence factors and their mode of action. Following the chronology of *Leptospira* invasion, they discuss first the role of adherence factors. Invasion comes next, where *Leptospira* stands out thanks to its abundance of metalloproteases. Subsequently, the authors discuss in detail the different toxins that come into play as soon as the pathogen has made its way into the host organism, notably its sophisticated tools to interfere with blood coagulation. Finally, Sun et al. list *Leptospira*'s attributes which are responsible for the massive pro-inflammatory response, and stress the importance of a better understanding of all the migratory features of *Leptospira* in order to stop it in time.

Original articles

Major Tom to Motoneuron: H-reflex depression happens at the pre-synaptic level

One of the very first medical experiments to be carried out on the International Space Station (ISS) was the effect of zero gravity on the Hoffmann's reflex (H-reflex) [38]. A short electrical stimulation of a peripheral nerve elicits first an efferent response (M-wave) towards the muscle, then an afferent one towards the corticospinal tract (H-wave), where a motor neuron is activated, leading to the contraction of the muscle located at the initial stimulation spot (H-reflex). In addition, the afferent signal also activates inhibitory interneurons, which dampen the signal before it travels along the motor neuron. Repetitive stimulation leads to the depression of the H-reflex, potentially in order to avoid over-stimulation of the α motor neuron and the muscle [39]. The system is frequently disrupted because of spinal disinhibition in various pathologies, such as diabetic neuropathy [40], or after spinal cord injury (SCI) [41], leading to spasticity and pain. Despite the large amount of related studies, it was still unclear whether the inhibition steps in at the pre- or post-synaptic level. Chang et al. decided to shed light on the exact mechanism by testing healthy subjects with a classic rate-sensitive H-reflex, patients with acute sensory-impaired SCI, and healthy individuals lacking the rate-sensitive depression. They combine testing for H-reflex depression with recording of motor evoked potentials (MEP) after direct α motor neuron excitation by transcranial stimulation in all three test groups. Observing no decrease in MEP in any group, the authors provide the most solid experimental evidence in humans that the H-reflex takes place at the pre-synaptic level [42].

The gland next door: does hyperparathyroidism impact on papillary thyroid carcinoma outcomes?

Thyroid cancer, affecting an endocrine gland whose hormonal secretions regulate metabolism and calcium homeostasis, is

on the rise worldwide [43]. Papillary thyroid carcinoma (PTC) is its most frequent subtype, and the standard treatment consists in surgical removal often followed by radioactive iodine therapy, with very good chances for remission [44,45]. Parathyroid glands in turn are located directly on the back of the thyroid, and in charge of regulating blood calcium levels via the secretion of parathyroid hormone (PTH). Excessive PTH production, or hyperparathyroidism (HPT), is mostly caused by benign adenomas, or the consequence of chronic kidney failure. The resulting perturbation of the calcium metabolism can cause various complications ranging from weakness to osteoporosis [46]. Given the physical proximity between the two glands, as well as some reports linking HPT with several cancers [47,48], Tsai et al. undertook to retrospectively investigate if an additional occurrence of hyperparathyroidism impacts PTC cancer prognosis in the long term. Their findings indicate that hyperparathyroidism occurs in slightly over 1% OF PTC cases, and has no statistically significant impact on non-remission risk. However, it correlates with an increased non-thyroid cancer related mortality in PTC patients, mainly due to cardiovascular events [49], which are possibly linked to the defective parathyroid function [50].

Mirror, mirror on the wall: FACE-Q survey validated for Mandarin Chinese

In the previous issue of the Biomedical Journal, researchers of the Chang Guang Memorial Hospital in Taiwan emphasized the importance to take into account the patient's self-perception before and after orthognathic surgery [51], given its impact on the individual's quality of life [52]. However, many aesthetic values are culture-dependent. Thus, this time, Su et al. ensure that the standard questionnaire "FACE-Q", used for auto-evaluation of many types of facial aesthetic surgeries, and originally written in English [53], meets both linguistic and psychological requirements after translation into Mandarin Chinese specifically for the case of orthognathic surgery. The authors confirm both the accuracy of the translation, the consistency of the system, and its usefulness in translating the psychological benefit from this type of surgery [54].

Turning the inside out: how trustworthy is virtual colonoscopy?

Colon polyps, small masses of cells on the lining of the colon, are mostly harmless. Nevertheless, some can develop into colon cancer, which ranks 3rd on the list of most frequent cancers, thus early detection by screening and subsequent removal are highly recommended [55]. CT colonography, also called virtual colonoscopy, is an x-ray based imaging procedure where three-dimensional pictures are reconstituted from two-dimensional cross-sections. Compared to conventional colonoscopy, the procedure allows for the visualisation of the entire colon, and is less invasive, discomfoting, risky and costly [56]. Yet it is still regarded with some suspicion, because it is less precise for small lesions, and sensitive to image distortion through movement, or computed analysis mistakes [57]. Subsequent image treatment includes the subtraction - or segmentation - of other organs, bones and fluid from the images [58]. Inaccuracies during this process could lead to measurement errors regarding polyp size and number, although this issue has never been thoroughly

investigated and quantified. For this reason, Manjunath et al. set out to methodically compare different volume-measurement techniques on available CT colonography datasets with and without segmentation. They come to the conclusion that in all cases, the segmentation proved actually robust and generated accurate results [59].

Try again – how much bad news are isolated local recurrence for HR + breast cancer?

Breast cancer remains the most common cancer in women,² and three quarters of the cases are hormone receptor positive (HR+), meaning that the tumour cells express the oestrogen and/or the progesterone receptor [60]. The reappearance of cancerous lesions close to the initial tumour site, termed isolated local recurrence (ILR), after surgery and postoperative therapy is *per se* bad news, as half of the cases tend to develop distant metastasis within five years [61]. Yu et al. aimed in their present study at determining further features that would prognosticate distant metastasis-free and overall survival (DMFS and OS) outcome in primary HR + patients with ILR. The authors compare the primary tumour characteristics, the histological consistency between the primary and recurrent tumours, as well as the surgical and postoperative treatments, and correlate them with the patients' outcome. They identify 29 months as the most appropriate cut-off for time to ILR to reflect survival. Moreover, primary tumour size and grading are confirmed to be predictors for DMFS and OS. In addition, Yu and colleagues encourage further studies aiming at incorporating novel agents into hormone therapy, which remains the first choice of treatment for ILR [62].

Brief communication

Rated PG-13: a severe case of facial necrotising fasciitis

"If there was ever a disease fit for a science-fiction writer, flesh-eating disease got to be it", wrote Peter Watts in 2011 on his blog,³ after contracting the disease more correctly called *necrotising fasciitis*. In this case, the fatal combination of breached skin, an underperforming immune system and various bacteria can sometimes lead to the infection, and subsequent necrosis of connective tissues, as well as images that are not for the faint-hearted [63].

In this brief communication, Chou et al. report a case compiling all textbook worst-case scenarios – an individual with untreated diabetes, and an advanced stage of necrotising fasciitis of the entire head and neck – with an astonishing good ending. The authors detail the initial findings, as well as all treatment steps that succeeded in saving the patient [64].

Conflicts of interest

The author declares no conflict of interests.

² <https://www.wcrf.org/dietandcancer/cancer-trends/worldwide-cancer-data>.

³ <https://www.rifters.com/crawl/?p=1831>.

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