BBA - Molecular Cell Research 1865 (2018) 831–841

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Review

Cancer cell cannibalism: Multiple triggers emerge for entosis

J. Durgan*, O. Florey

Babraham Institute, Cambridge, UK

ARTICLE INFO

Keywords:
Entosis
Cell cannibalism
Mitosis
Mitotic rounding
Cdc42
Glucose
AMPK

ABSTRACT

Entosis is a form of epithelial cell engulfment and cannibalism prevalent in human cancer. Until recently, the only known trigger for entosis was loss of attachment to the extracellular matrix, as often occurs in the tumour microenvironment. However, two new studies now reveal that entosis can also occur among adherent epithelial cells, induced by mitosis or glucose starvation. Together, these findings point to the intriguing notion that certain hallmark properties of cancer cells, including anchorage independence, aberrant proliferation and metabolic stress, can converge on the induction of cell cannibalism, a phenomenon so frequently observed in tumours. In this review, we explore the molecular, cellular and biophysical mechanisms underlying entosis and discuss the impact of cell cannibalism on tumour biology.

1. Entosis and cell cannibalism in physiology and disease

Entosis is a specialised form of cell engulfment, its name derived from the Greek word 'entos', meaning inside, into or within [1]. During entosis, one live and viable epithelial cell is completely internalised by another, to form a so-called 'cell-in-cell' structure (Fig. 1A, B). Typically, the internalised cell is then killed and digested by its host in an unusual act of cellular cannibalism [1,2]. Cell-in-cell structures have long been observed in tumour samples [3] (Fig. 1C), but the underlying mechanisms and functional consequences of this process remain to be fully understood. Intriguingly, various forms of cell cannibalism also occur physiologically, from bacteria to mammals [4], suggesting this primeval form of cell killing and feeding has been conserved across evolution. In the following sections, we explore the concept of cell cannibalism in physiology and disease.

1.1. Cellular cannibalism across evolution

Cell cannibalism refers to the engulfment and digestion of one cell by another. This process is harnessed by sporulating bacteria [5] and predatory amoebae [6,7] to avoid starvation. A similar phenomenon can also be observed among the cells of metazoans, often in association with non-apoptotic cell death processes. For instance, cell engulfment contributes to cell death events during *C. elegans* development [8–10] and in *Drosophila* nurse cells [11]. In mammals, cell-in-cell structures have been extensively documented by pathologists, and can occur in various configurations [3,12–14], including thymocytes in thymic nurse cells [13], immune cells in megakaryocytes [3] or tumour cells [13,15], or tumour cells in other tumour cells [1]. As such, cell cannibalism represents an ancient process that has been conserved across evolution and in a range of different contexts.

1.2. Mechanisms of cell engulfment

Phagocytosis represents the best-characterised form of macroscale cell engulfment and digestion [16]. In addition to eliminating pathogenic bacteria and fungi, phagocytosis can target dead, dying or diseased cells, clearing unwanted debris and mediating fundamental immune functions [17]. Mechanistically, phagocytosis is an active process, driven by the engulfing 'host' cell, which can be either a professional (e.g. neutrophil) or non-specialised phagocyte (e.g. epithelial cell), in response to signals from its prey [16,18].

In contrast to phagocytosis, other forms of cell engulfment can target viable cells for internalisation [3], which is often followed by their execution and digestion. Multiple modes of live cell engulfment and killing have been described, including: phagoptosis (e.g. neurons internalised by glia) [18]; suicidal emperipolesis (e.g. T-cells in hepatocytes) [19], emperitosis (immune killer cells in tumour cells) [20], cannibalism (e.g. lymphocytes in metastatic melanoma cells) [21], homotypic cell cannibalism (pancreatic adenocarcinoma cells) [22] and entosis (epithelial cells) [1]. These processes all involve 'non-professional' host cells, that are not specialised for engulfment, and proceed through a variety of somewhat different internalisation mechanisms [23]. These mechanisms, and the diverse biological processes with which they are associated, remain to be fully understood, forming the basis of an emerging new field of 'cell-in-cell' research [14].

* Corresponding author.
E-mail address: Joanne.Durgan@babraham.ac.uk (J. Durgan).

https://doi.org/10.1016/j.bbamcr.2018.03.004
Received 31 January 2018; Received in revised form 2 March 2018; Accepted 6 March 2018
Available online 14 March 2018

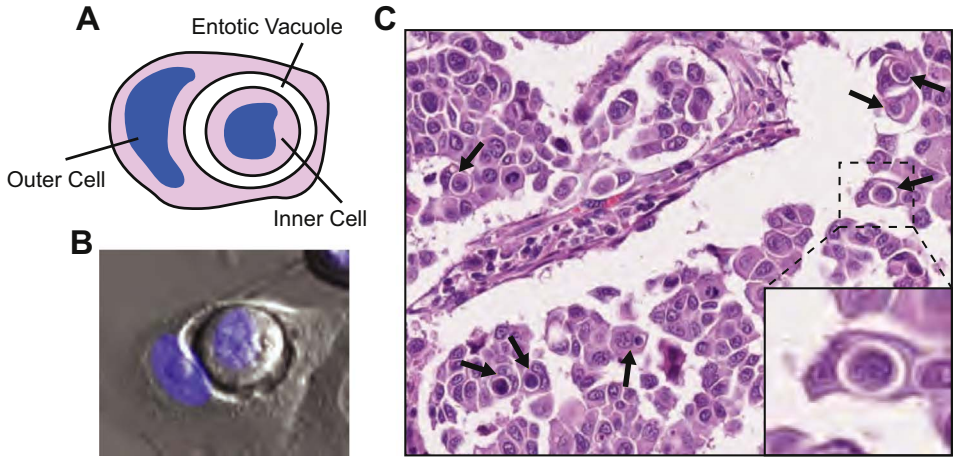

Fig. 1. Entosis: epithelial cell-in-cell formation. A) During entosis, one live and viable epithelial cell is completely engulfed by another, leading to the formation of a 'cell-in-cell' structure. The internalised cell is housed within an entotic vacuole in the host cell cytoplasm. B) An entotic cell-in-cell structure. Live MCF10A cells with nuclei stained in blue, imaged using DIC microscopy; a representative cell-in-cell structure is shown. C) Cell-in-cell structures in a breast carcinoma sample, stained with H&E. Arrow heads indicate cell-in-cells, the inset shows an enlarged image of one such structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1.3. Entosis: homotypic live epithelial cell invasion and cannibalism

Entosis represents a distinctive form of cell engulfment, death and cannibalism that occurs specifically between live epithelial cells [1]. During entosis, one live and viable epithelial cell is completely internalised by another. The engulfed cell is housed inside a large vacuole within the host cell cytoplasm (Fig. 1A, B), where it can then be killed and digested. Given this mechanism, entosis bears several important distinctions from phagocytosis. Firstly, the entotic host cell is not a professional phagocyte, but a normal epithelial cell, which can internalise a neighbour under certain conditions. Secondly, the target cell is live and viable, bearing no morphological or functional signs of apoptosis, necrosis or cell damage [1,24,25]; indeed, internalised cells can even undergo cell division inside of the entotic vacuole, a definitive demonstration of viability [1]. Finally, internalisation is driven by biophysical forces produced by the internalising cell, rather than by its host, representing a form of cell invasion, rather than active engulfment [1]. As such, entosis represents an intriguing and distinctive form of cell cannibalism.

1.4. Entosis in cancer

Epithelial cell-in-cell structures have been frequently observed in cancer samples for more than a century [3,12,26]; for instance, Leyden's 1904 observations describe whole tumour cells, housed inside vacuoles within other tumour cells, with the appearance of a 'bird's eye' [3]. Entosis has since been documented in a wide range of carcinomas, derived from different tissues of origin, including breast (Fig. 1C), cervix, colon, stomach, liver, melanoma, head-and-neck, and small cell carcinoma of the lung [1,27–31], occurring at a rate of 0.3–2.5% of the total sample population [1]. Cell-in-cell structures are especially common in fluid derived samples, for instance, in pleural effusions [32], ascites (mouse Ehrlich ascites carcinoma) [33], urine [32,34,35] and metastatic breast cancer fluid exudates, where frequency is scored at 1–20% [1]. As such, entosis represents a well-established and relatively common occurrence in tumour biology, which, surprisingly, has not been widely studied, and remains to be fully understood in terms of both mechanism and possible clinical value.

1.5. Entosis-like processes in physiology

While entosis has been best documented in the context of cancer, similar processes also occur in normal physiology. For instance, during early pregnancy, uterine epithelial cells are internalised by blastocyst trophoblast cells, to facilitate embryonic implantation, in a cell invasion process that closely resembles entosis [36]. Similar cell engulfment events can also occur between pairs of blastomere cells [37], indicating that entosis could potentially occur within the embryo, and may be worth screening for during development. It seems likely that additional examples of physiological cell-in-cell formation await discovery, and that entosis may represent a normal biological process that is subverted in cancer.

2. Molecular, cellular and biophysical mechanisms of entosis

Mechanistically, entotic cell cannibalism is a complex process that encompasses some fascinating cell biology. Entosis begins with the formation of epithelial adherens junctions, and the associated generation of actomyosin-contractility, which together drive cell engulfment [1]. Once internalised, the inner cell is typically killed and digested by its host, through a mechanism involving non-canonical autophagy, lysosomes and nutrient recovery [2]. Entosis thus depends upon dynamic cytoskeletal changes and biophysical forces during cell-in-cell formation, and novel signalling and degradative pathways during cell killing and digestion. Each step of this intriguing process is examined in more detail below.

2.1. Initiation of entosis: multiple triggers for cell cannibalism

Entosis was first discovered under conditions of matrix deadhesion, with the seminal observation that cell-in-cell structures form when MCF10A breast epithelial cells are cultured in suspension [1], or similarly, during the early stages of spreading [38]. Along with matrix detachment, this process requires adherens junction formation and the accompanying generation of actomyosin contractility [1,39] (Fig. 2, Table 1). The current working model proposes that this junctional contractile force is usually opposed by integrin engagement [1], in an apparent 'tug-of-war' between cell-cell and cell-matrix contacts, similar to that observed during cell scattering [40]. However, matrix deadhesion can render this force unopposed. If an imbalance in contractility exists between the two cells of a suspended pair, one cell may then push into the other (Fig. 2). Consistent with this model, entosis is prevalent in cancer, where matrix deadhesion and anchorage independence are common features [41]. Within tumour samples, cell-in-cell structures are often found in regions distal to the basement membrane, that stain negative for matrix components [1], and are particularly prevalent in suspended samples, such as fluid exudates, urine and bile [3].

Importantly, entosis has now also been observed among fully adherent epithelial cells [24,31,42,43], indicating that cell-in-cell formation can involve profoundly different mechanisms, and occur in a wider range of contexts than previously appreciated. Two new studies have identified mitosis [31] and glucose starvation [43] as additional triggers for entotic cell cannibalism, opening important new lines of investigation and significantly broadening this field. Strikingly, the