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Review

Arrested development and the great escape – The role of cellular senescence in pancreatic cancer

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ABSTRACT

The outcomes of pancreatic cancer remain dismal due to late clinical presentation and the aggressive nature of the disease. A heterogeneous combination of genetic mutations, including KRAS, INK4a/CDKN2A and p53, underpin the propensity of pancreatic cancer to rapidly invade and disseminate. These oncogenes and tumour suppressors are strongly associated with cellular senescence, therefore suggesting this process as having a key role in malignant transformation. In the context of cancer, oncogenic stimuli trigger the senescent phenotype resulting in cell cycle growth arrest and prevention of progression of premalignant lesions such as PanINs. However mutations of the aforementioned oncogenes or tumour suppressors result in cells escaping senescence and thus allowing tumours to progress. This review presents current evidence regarding both senescence induction and escape with respect to pancreatic cancer, highlighting the key roles of p19ARF, p53, Rb and P16INK4a. The epigenetic regulatory component is also discussed, with relevance to DNA methylation and HDACs. Lastly the role of the tumour microenvironment, and in particular pancreatic stellate cells, is discussed with regards to the induction of a senescence associated secretory phenotype (SASP), with SASP-associated secretory factors contributing to the pro-tumorigenic effects of the surrounding activated stroma. Further work is required in this field to elucidate the most important pathways relating to cellular senescence that contribute to the belligerent nature of this disease, with the aim of discovering therapeutic targets to improve patient outcomes.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer, with over 8000 new cases diagnosed each year in the UK, and outcomes are extremely poor (UK, 2014). It is one of the five most lethal malignancies accounting for 5% of total cancer deaths in the UK with a 5-year survival rate of only 3%. The poor prognosis is in stark comparison to other types of cancer; 5 year survival in breast cancer is 80% and in prostate cancer is 70%. Survival rates have remained unchanged over the last 40 years despite advances in chemotherapy regimens (FOLFIRINOX)

Abbreviations: ARF, alternative reading frame; αSMA, alpha smooth muscle actin; CDK, cyclin-dependent kinase; DDR, DNA damage response; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; FGF, fibroblast growth factor; HDAC, histone deacetylase; HIC1, hypermethylated in cancer-1 gene; MMP, matrix metalloproteinase; ncRNA, non-coding RNA; OIS, oncogene induced senescence; PanIN, pancreatic intra-epithelial neoplasia; PaSC, pancreatic stellate cells; PcG, polycomb group; PDAC, pancreatic ductal adenocarcinoma; PDGF, platelet derived growth factor; Rb, retinoblastoma; SaβG, senescence-associated β galactosidase; SAHF, senescence associated heterochromatic foci; SASP, senescence-associated secretory phenotype; TGFβ, transforming growth factor beta.

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(Conroy et al., 2011). Therefore, taken together with the difficulty in diagnosis and our poor understanding of the important initiating molecular and cellular events surrounding the development of pancreatic cancer, it is not surprising there is a lack of effective therapeutic targets.

Cellular senescence was first postulated over 50 years ago when it was observed that the extent to which a cell can divide and proliferate is limited by this form of irreversible cell cycle arrest. Over time eventually all normal human somatic cells stop dividing and enter this state of replicative senescence, in order to maintain a balance between differing tissue and cell types; however during this period they remain viable (Hayflick, 1965; Campisi and d'Adda di Fagagna, 2007; Kuilman et al., 2010; Collado et al., 2007). This process also occurs in response to the development of premalignant cells, such as pancreatic intra-epithelial neoplasia (PanINs), and therefore acts as a defence mechanism to prevent cancerous transformation. However, in the presence of a complex combination of genetic mutations, these cells may escape senescence, resulting in tumour progression. Senescent changes also impact on the surrounding tumour microenvironment, also termed stroma, which consists of a wide variety of inflammatory, vascular and neural components which interact with tumours resulting in a desmoplastic reaction, encouraging tumour invasion, dissemination, and chemoresistance (Maitra and Hruban, 2008; Bissell and Radisky, 2001).

Understanding senescence, and potentially identifying related pathways that may be targeted with therapeutics, is of particular relevance to PDAC, as this phenomenon has been linked to both chronic inflammation and the malignant transformation of PanINs, both of which are key precursors to tumour development. Furthermore PDAC shows the most abundant stromal activity as compared to other cancers (Vonlaufen et al., 2008); therefore senescence may be playing a key role in the aggressive nature of this disease, with particular relevance to cancer associated fibroblasts (pancreatic stellate cells – PaSCs) which have such a prominent role in the tumour microenvironment. This review examines the current evidence regarding the role of cellular senescence and associated oncogenic stimuli as both a protective and detrimental process in the pancreas.

2. Cellular senescence

Senescence is an anti-cancer mechanism resulting in cell cycle growth arrest as a result of either physiological or oncogenic stress (Campisi, 2011). Whilst this process was previously only linked with tumour suppression and ageing, more recent studies have demonstrated its role in normal embryological development, suggesting this as the evolutionary origin of damage-induced senescence (Storer et al., 2013; Muñoz-Espín et al., 2013; Sherr and DePinho, 2000; Serrano et al., 1997). The senescent phenotype by no means results in reduced cell activity, but rather these cells remain metabolically active, communicating with each other and the surrounding microenvironment in an attempt to both prevent tumour progression, and mobilise a local and systemic response. Characteristics of senescent cells are highlighted in Table 1.

Replicative senescence occurs following repeated mitotic divisions, resulting in shortened telomeres (the protective caps at the end of chromosomes) which ultimately reach their "Hayflick limit" (Hayflick, 1965). Alternatively the term premature senescence has been coined, which refers to the presence of senescence (induced by a variety of conditions including cellular stress and oncogenic stimuli) without telomere disruption (thus premature) (Sherr and DePinho, 2000; Serrano et al., 1997).

The most common event leading to the development of the senescent phenotype is loss of telomeric DNA (Frias et al., 2012).

Table 1
Characteristics of senescent cells.

Characteristic	Detail
Morphology	Large flattened cells, with vacuole rich cytoplasm and large nuclei (DeNicola and Tuveson, 2009).
Chromatin structure	Formation of SAHFs (senescence associated heterochromatic foci) – a distinctive type of facultative chromatin structure, with heterochromatin proteins, that accumulates in senescent fibroblasts and controls the stability of growth arrest (Narita et al., 2003).
Biomarker expression	Senescence-associated β galactosidase (SABG) has been shown to be the most reliable and specific detector of senescence in pancreatic precursor lesions (Caldwell et al., 2012).
Molecular pathway involvement	Various oncogene-induced tumour suppressor proteins (p16INK4a and p19ARF) and associated loci (CDKN2a) (Bardeesy et al., 2006). p53 and retinoblastoma activation (Vogelstein et al., 2000; Romagosa et al., 2011).
Senescence associated secretory phenotype (SASP)	Cells develop altered secretory activity whilst in a senescent state, resulting in a protumorigenic effect through an increase in proteins, interleukins, growth factors and proteases within the tumour microenvironment (Coppé, 2008; Penfield et al., 2013).

Telomeres protect the ends of linear chromosomes by forming a capping complex with DNA repeats and associated proteins (de Lange, 2002). This both prevents DNA degradation, and distinguishes the ends of chromosomes from DNA breaks. Repeated cellular division results in shortening of telomeres, and this can ultimately lead to disruption of the capping complex and a subsequent DNA damage response (DDR). This mobilises various pathways resulting in the induction of senescence and arrest of the growth cycle (di Fagagna et al., 2003).

Telomerase is the key enzyme involved in maintenance and regulation of telomeres, and allows cells to avoid senescence and subsequently extend their lifespan significantly (Bodnar et al., 1998). Therefore unsurprisingly their expression is heavily linked to cancer (detected in 85% of cases), allowing cells to undergo unlimited proliferation (Shay and Bacchetti, 1997; Hahn et al., 1999). Telomerase inhibition thus represents a promising treatment approach, with a recent in vitro pancreatic cancer cell line study demonstrating the telomerase inhibitor Imetelstat (GRN163L) resulted in cancer cell crisis through activation of the DDR, with evidence of senescence induction (shortened telomeres) and apoptosis (Burchett et al., 2014). Potential issues with this approach include telomere stabilisation following initial shortening and prolonged delays before cell crisis, suggesting treatment regimens require optimisation prior to future trials, which could be particularly beneficial as an adjuvant therapy.

Telomere shortening can occur in any mitotically dividing cell, and thus is an essential part of ageing. However senescence may also be stimulated by the absence of telomeric alterations, through cellular stress or oncogene activation, as well as an alternative lengthening telomere mechanism; these various mechanisms will now be discussed in further detail.

3. Senescence induction

As mentioned, replicative senescence occurs in all cells as a result of telomere loss, and is strongly associated with ageing.