Pancreatic carcinoma development: new etiological and pathogenetic evidence

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ABSTRACT

Pancreatic adenocarcinoma (PAC) is a very aggressive cancer with a poor prognosis. To date, the causes and pathogenetic mechanisms involved in the development of this malignancy remain largely unknown. Therefore, additional studies are required to improve our knowledge of the events that occur during the process of pancreatic carcinogenesis. The purpose of this article is to describe the most recent evidence, concerning the possible risk factors and mechanisms that may contribute to determine the development of PAC, as well as models, such as the tensegrity model, that may explain this complex process. Available studies suggest that approximately 15-20% of human malignancies are somehow associated with chronic infection. Some epidemiological research has shown that some infectious agents represent risk factors for PAC. In particular, several reports showed that the infection caused by some micro-organisms, including helicobacter pylori and some bacterial species of oral microbiota, as well as by viral agents, such as human immunodeficiency virus (HIV), and hepatitis B (HBV) and C (HCV) viruses, is associated with an increased probability of developing PAC. For the first time, observational studies and meta-analyses have suggested that HBV and HCV, two hepatotropic viruses with oncogenic properties, may be also risk factors for PAC. However, the small number of available reports, nearly all performed in Asian populations, limits their validity to these ethnic groups. Therefore, additional studies focusing on populations of different geographical areas and enrolling larger series of patients are required to confirm this association. Furthermore, an accurate description and a better understanding of the events and of the pathogenetic mechanisms involved in the process of pancreatic carcinogenesis, as proposed by the tensegrity model, might be a useful approach to effectively deal with this pathology.

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Introduction

Pancreatic adenocarcinoma (PAC) represents a very aggressive and highly fatal malignancy, with an overall 5-year survival of less than 5%.1 It is the 8th most frequent cause of cancer-related death, and it causes approximately 250,000 deaths worldwide every year.2 The only treatment that may improve patients’ survival is represented by a complete surgical resection with associated adjuvant chemo- or chemo-radiotherapy.3,5 Unfortunately, although in recent years better diagnostic and therapeutic procedures have been introduced into clinical practice, current 5-year survival after curative resection remains very low (approx. 15-20%).6 Advanced stage at presentation, aggressive disease, early metastatic dissemination, absence of precocious symptoms and signs of disease, and lack of effective systemic therapies,
make the diagnosis and treatment of this cancer very difficult. At the time of diagnosis, surgical resection with a curative intent is possible only in a minority of PAC patients.

Therefore, there is a pressing need for research and development of new diagnostic and therapeutic approaches to improve the management and the poor prognosis of patients with this malignancy. Etiological factors and pathogenetic mechanisms involved in the process of carcinogenesis and, in particular, the causes of PAC development, are still largely unknown. In particular, only cigarette smoking and family history of this neoplasm are well-known risk factors for PAC. Furthermore, heavy fat intake in the diet, alcohol abuse, as well as history of diabetes mellitus and chronic pancreatitis have been associated with a higher incidence of PAC.

Evidence from various epidemiological and basic research studies suggests that approximately 15-20% of all human cancers are somehow linked to a persistent infection. In recent years, the potential role of some infectious agents, both bacteria and viruses, as risk factors for pancreatic carcinogenesis has been described with increasing frequency. In particular, with regards to bacteria-related infections, a recent meta-analysis, including six observational studies performed in the last decade, suggested that *Helicobacter pylori* infection is associated with an increased risk of developing pancreatic cancer. It has already been demonstrated in the past that this bacterium is involved in the pathogenesis of peptic ulcer disease as well as of gastric carcinoma and lymphoma. Further studies suggested a possible link between oral pathologies, such as periodontitis, and enhanced risk of pancreatic cancer. There have also been reports of an association between variations in patients’ oral microbiota (in particular, some species of salivary microorganisms, such as *Neisseria elongata*, *Streptococcus mitis*) and a higher incidence of pancreatic diseases, including neoplasia of this organ. Nevertheless, it is still unclear whether oral flora has a causative or reactive role in carcinogenesis of pancreas.

In addition, some authors showed a significantly higher risk of PAC development in patients with HIV-related infection in comparison with the general population during highly active antiretroviral therapy, although other studies showed no increased incidence of this carcinoma in HIV-positive subjects undergoing this treatment. Several very recent epidemiological studies and meta-analyses have suggested that hepatitis B (HBV) and hepatitis C (HCV) virus, two hepatotropic viruses with well-known oncogenic properties may be risk factors not only for liver carcinoma, intrahepatic cholangiocarcinoma and for some forms of non-Hodgkin’s lymphoma, but also for PAC. HBV and HCV persistent infections represent a serious public health problem worldwide, affecting approximately 400 and 180 million people, respectively. It is widely recognized that in the liver both viruses may cause a persistent local necroinflammatory disease, with different patterns of severity and disease course, and that antigens and replicative sequences of HBV and HCV have also been detected in different extra-hepatic tissues, including pancreas.

The aim of this paper is to report recent insights into the role of HBV and HCV in pancreatic carcinogenesis. Particular attention is focused on a description of the following issues: i) studies and recently published meta-analyses assessing the possible association between HBV or HCV and risk of pancreatic cancer; ii) new models concerning cytopathogenesis and histogenesis of this carcinoma. Early studies reported that PAC develops from cells of ductal epithelium. Nevertheless, more recent evidence suggests that at least a part of human and animal PAC may have an acinar/centroacinar origin or develop in specialized structures, the so-called gland-like mucinous outpouches of major ducts; iii) pancreatic microenvironment during development of cancer in this organ. The transition from normal pancreatic tissue to cancer is characterized by a dynamic network and cooperative interactions, occurring among cells with a progressively transformed phenotype, immune, endothelial, pancreatic stellate cells, extracellular stroma and modulating mediators, such as cytokines, inflammatory and growth factors; iv) the *tensile integrity or tensegrity* model. This refers to a key characteristic that all nucleated cells present, *i.e.* that they contain a special structure, known as the cytoskeleton, a molecular scaffold that generates tensile forces and distributes them to other cellular and extracellular components. Normal tissue function and cellular shape stability are maintained by regular and constant architectural connections, including cell-cell and cell-stroma adhesions. In this way, cells may control their architecture and form. It is well known that some important cellular functions, including cellular differentiation, apoptosis or growth, are modulated by cellular architecture and shape. Some experimental studies suggested that the deregulation of tissue structural context and of mechanical properties of cells and extracellular matrix causes changes in intracellular biochemistry and gene expression; therefore, these events may promote neo-plastic transformation and tumor progression; v) a description of the available *in vivo* and *in vitro* studies of the immune system response against pancreatic cancer and, in particular, the possible role of T-regulatory (Tregs) cells during the process of carcinogenesis in this organ.
Possible role of hepatitis B and C virus as risk factors for pancreatic carcinoma

Several studies showed that HBV- and HCV-related persistent infection is able to cause a necroinflammatory liver injury with different patterns of severity and course. Chronic hepatic damage, induced by both viruses, represents a high-risk condition for cirrhosis and hepatocellular carcinoma (HCC) development. On the other hand, even if clinical evidence suggests that HBV and HCV may infect pancreas and viral antigens, as well as the fact that replicative intermediate forms have been detected in pancreatic specimens, to date only a few studies investigating the role of both viruses as risk factors for pancreatic carcinogenesis in humans are available. There are several reasons for the small number of reports on this topic: i) difficulties in studying pancreas by means of modern imaging techniques or biopic procedures, because of the anatomic localization of this organ in the retroperitoneal space; ii) the small size of the majority of precursor cancerous lesions that often prevents their detection at early stages; iii) life-cycle characteristics of both viruses, making it difficult to assess their role in human pathology; iv) ability of HBV and HCV to productively infect, in vitro, a narrow range of hepatic cellular lines and, in vivo, only humans and chimpanzees.

However, in recent years, this subject has gradually gaining importance and some studies have already been conceived and completed or are still ongoing. It is now well-known that, in patients with persistent HBV- and HCV-infection, antigens and genome sequences of both viruses are detectable not only in liver, but also in extra-hepatic tissues. The first studies concerning this topic date back to 1980-1981 when hepatitis B surface antigen (HBsAg) was observed in pancreatic juice and bile of patients with both acute and chronic virus-related hepatitis, whereas HBsAg and hepatitis B core antigen (HBcAg) were found in the cytoplasm of pancreatic acinar cells in individuals with different forms of hepatic damage on whom a post mortem examination was performed. In 1984, a study showed the presence of some integrated HBV-DNA sequences in pancreatic tissue of 2 patients who died of HBV-related liver diseases. In 1985, a retrospective study was performed on pancreatic specimens of 199 patients undergoing a surgical procedure and detected HBsAg in the pancreatic acinus epithelia and small ductules in 2 patients with chronic pancreatitis and in 5 subjects with pancreatic adenocarcinoma.

It has been shown that HCV is also able to replicate in extra-hepatic sites. HCV antigen and its replicative forms have been observed in several organs, including lymph nodes, spleen, ovary, thyroid, uterus and pancreas. In spite of these initial observations, it is only in the last five years that epidemiological trials, including case-controls and prospective studies, have been performed to assess the possible association between previous or persistent HBV- or HCV-related infection and risk of pancreatic carcinoma. Most of these studies have been published between 2010 and 2012, and for the most part they were carried out in China. In September 2012, a systematic review of observational studies was published that focused on HBV/HCV status and risk of malignancy in pancreas. In this study, the authors hypothesized that pathogenetic mechanisms involved in HCC development might be common to pancreatic carcinogenesis.

Subsequently, between the end of 2012 and the beginning of 2013, at least three meta-analyses have been published confirming that both viruses are risk factors for the development of pancreatic cancer.

Further evidence supports the results of these epidemiological studies.

- Several in vitro studies demonstrated that some human hepatoma cells and pancreatic cancer cell lines were able to undergo a process of transdifferentiation into hepatocyte-like cells upon definite and well-standardized conditions of culture. A wide series of induction programs have been proposed, on the basis of different experimental conditions and objectives. In particular, upon treatment with dexamethasone and oncostatin M, cellular lines from a pancreatic origin were able to trans-differentiate into permissive hepatocyte-like cells that were able to sustain HBV replication with production of viral antigens, replicative forms and entire genomes, when they were permanently transfected with HBV-DNA. Chronic pancreatic damage might induce in this organ the transdifferentiation of some cells into hepatocyte-like cells, supporting HBV replication.

- On the basis of research on human and animal morphogenetic processes, liver and pancreas have in common many features during their embryological growth, arising from common multipotent cells of endoderm origin.

- Some intracellular signaling paths, deregulated during pancreatic carcinogenesis, are similar to those perturbed in patients with chronic HBV or HCV infection with associated persistent hepatic inflammatory injury and HCC development.

Pancreatic carcinoma cytogenesis and histogenesis

Available data suggest that, as reported in liver, development of pancreatic carcinoma results from a multistep process. Consensus to the nomenclature and classification system of human pancreatic cancer precursors and identification of related genome mutations substantially improved our understanding of this malignancy. It has been suggested that PAC develops...
from early lesions, such as pancreatic intraepithelial neoplasia (PanINs), mucinous cystic neoplasm and intraductal papillary-mucinous neoplasm. Among these pre-malignant conditions, PanINs are the best known. In particular, they are characterized by a wide spectrum of patterns, ranging from low- (PanIN-1 A and 1B) to high-grade (PanINs-2 with dysplasia, PanINs-3 or carcinoma in situ, invasive cancer) lesions. However, although in the past a model of linear progression, from PanINs-1 to PanINs-3, has been proposed to explain how pancreatic cancer develops, no definite demonstration has been obtained to validate this paradigm. The sequence of steps, starting from pre-neoplastic lesions and leading to invasive malignancy, might follow a different course.\textsuperscript{51-54} It has to be emphasized that neither the cells from which pancreatic carcinoma originates, nor its histogenesis, are completely understood, and no univocal conclusions have yet been obtained. Early research reported that this cancer arises from cells of epithelial duct. Nevertheless, recent studies report that at least a part of pancreatic carcinoma may originate from acinar or centro-acinar cells or develop in specialized pancreatic compartments, represented by the gland-like mucinous outpouches of major ducts.\textsuperscript{55,56} In particular, available experimental evidence seems to suggest that, in a context of persistent inflammation in pancreatic tissue, a process characterized by acinar-to-ductal mucinous metaplasia may occur. Furthermore, in this organ, human acinar cells adjacent to areas of cancer present ductal markers.\textsuperscript{57} Metaplasia is a condition linked to an increased risk of cancer, because it induces a permissive setting where several pro-oncogenetic factors may be expressed.\textsuperscript{58} As a consequence of tissue damage, restoration of its normal structure is the consequence of a resultant regenerative process. Metaplasia is characterized by the substitution of one cell type with another. In particular, different mechanisms may be involved, such as selective replacement or expansion of cellular subsets, differentiation of progenitor cells, as well as transdifferentiation. This process is characterized by the transition between different types of differentiated cells.

Several cellular types, such as neoplastic, immune (macrophages, neutrophils, dendritic cells, effector and regulatory lymphocytes), pancreatic stellate, endothelial, bone-marrow derived cells, as well as modulating factors, including interleukins, cytokines, growth and inflammatory factors, contribute to this complex process. A dynamic crosstalk develops in this microenvironment where malignant and stromal cells interact and activate one another. One of the effects of this interplay is represented by the progressive deposition of a modified extracellular matrix (ECM) which surrounds cancer cells and includes collagen type I, III, V, fibrinogen, fibronectin. This tissue consists of a dense abnormal stroma, called desmoplasia, which is characterized by an increased stiffness.\textsuperscript{59,61}

Several mediators and cellular signaling pathways are involved in cancer initiation, progression and growth, inducing a process of inflammation and neovascularization in local tissue.\textsuperscript{52,63}

Cancer is a disease characterized by a deep deregulation of mechanisms, controlling organization of both cells inside the tissues and tissues within organs.\textsuperscript{64} Uncontrolled cellular growth is a necessary but not sufficient condition for cancer development. Neoplastic transformation of a tissue is characterized by an autonomous cellular proliferation, associated with a progressive disorganization of its normal structure as well as with the possibility of generating metastases. But a key event in this process is represented by the breakdown of proper epithelial-mesenchymal interactions. Several points have to be considered.

- **Cellular shape** is able to influence and modulate a large series of cellular activities, such as growth, differentiation, apoptosis, motility and ability to adhere to basal membrane and to tissue ECM.\textsuperscript{65}
- **Cytoskeleton** is the most important mechanical component of the cells. It includes three major interconnected elements: microfilaments, intermediate filaments and microtubules. On the basis of different intracellular organization and context, these structures may have a dual role and exert compression or tension. In particular, within cells, tensile forces generated by microfilaments and intermediate filaments are counteracted and balanced by forces, resisting compression, which are originated by microtubules and extracellular matrix adhesions.\textsuperscript{66}
- **Within cells**, tensile forces generated by microfilaments and intermediate filaments are counteracted and balanced by forces, resisting compression, which are derived from microtubules and extracellular matrix adhesions. Therefore, the cytoskeleton represents a dynamic scaffold that controls cellular stability and actively contributes to determine cellular shape. Furthermore, each cell must not be considered as a structure apart, but it is connected with

**Pancreatic microenvironment and carcinogenesis: tensional integrity or tensegrity model**

A progressive transformation from normal tissue to acinar-ductal metaplasia and to invasive cancer characterizes pancreatic carcinogenesis. A reciprocal interplay occurs among cells that are, step by step, acquiring a neoplastic phenotype and the adjacent stroma. Malignant cells release growth factors that, via paracrine and/or autocrine pathways, are able to modify and remodel surrounding connective tissue, promoting a supportive microenvironment for cancer initiation and growth.
adjacent cells and with neighboring ECM either directly or by means of a basal membrane (BM). As a whole, these different tissue components constitute a single and intricate, but perfectly integrated, framework.64

In order to explain the balance between intracellular and extracellular forces, several years ago, Donald Ingber suggested the application of a tensional integrity or tensegrity model. Therefore, mechanical loads act as developmental regulators.67,68 According to this model, cells may be considered as tensed structures that are resistant to shape distortion and that self stabilize by encompassing other supportive structures that counteract compression. Furthermore, cytoskeleton is directly associated with integrins, which represent specialized mechanoreceptors on the cellular surface. They are able to sense mechanical signals that are applied to cells and to react against them, generating different types of response, such as an increase in cytoskeletal tension. Mechanical distortion of cells, via integrin receptor system, results in alteration of cytoskeleton as well as of ECM. On the other hand, an increase in the exogenous tension (matrix rigidity or stiffness) produces also dramatic effects on intracellular signaling, on matrix adhesion, as well as in endogenous tension (contractility). On the whole, also these events may influence cellular fate, by switching cells among different states critical for cancer development, such as proliferation, apoptosis, differentiation and motility. Mechanical forces might act as regulators of cell and tissue development. Cytoskeleton represents a framework which controls and influences motility and function of intracellular organelles as well as proper activity and orientation of enzymes and substrates, with a key role in critical biochemical reactions within cells.69

Some experimental studies suggest that perturbation of tissue structure, as well as alteration of cellular and ECM mechanical properties, cause modifications of multiple intracellular signaling pathways. The final effect of these events is represented by a substantial change in intracellular biochemical activity and genome expression.70 For example, collective interactions occurring in an epithelial tissue among cytoskeletal structures mediate the adhesion of each cell to BM, influencing its form and modulating intracellular transduction of mechanical forces applied to tissue.71

Dynamics of immune response during the development of pancreatic cancer in animal models

A complete analysis of inflammatory response and desmoplastic reaction, involved in early as well as in more advanced phases of pancreatic carcinogenesis, has been possible only in animal models. A recent study assessed in vivo the dynamics of immune system activity during this process. In particular, the events that occur in pancreas during appearance and growth of this cancer have been reproduced in a genetically engineered mouse model that summarizes clinical, molecular and histological characteristics of this malignancy during its development from precursor lesions to invasive tumor.55,72

According to this model, several steps characterize the initiation and progression of this cancer, including: i) a progressive inflow of fibroblasts, stromal cells and leukocytes, gradually surrounding precursor lesions, during PanINs development; ii) production and release of an altered ECM, rich in collagen component, with characteristics similar to desmoplastic reaction detectable in cancer of pancreas. This process is modulated by pancreatic stellate cells (PSCs).

As a result of an acute or chronic injury in this organ, they become active and acquire a fibroblast-like phenotype. The most important features of activated PSCs include: vitamin A droplet loss, increase of mitotic index, alfa-smooth muscle actin production, enhanced motility and contractility.73,74 Pancreatic tissue is infiltrated by immune cells, which, on cellular surface, present leukocyte common antigen 45 (CD45). They progressively increase during development and progression of this malignancy. In peritumoral stroma, a small number of CD4+ and CD8+ T lymphocytes with anti-cancer effector properties is detectable in the early phases of the neoplastic process and these subsets do not posses activation markers. In advanced stages, an increased number of T lymphocytes infiltrating pancreatic tissue are observed, but these cells exhibit poor or no protection against cancer cells. A significant inflow of immune cells with immunosuppressive activities may be already observed in the early stage of pancreatic carcinogenesis. Phenotypic assessment of these immune cells shows that stromal tissue adjacent to precursor and invasive lesions is rich in Tregs cells, tumor-associated macrophages and myeloid suppressor cells.

Immune response in patients with pancreatic carcinoma: role of T-regulatory cells

In recent years, a large series of studies focused on how the immune system may counteract tumor promotion, initiation and progression. It is now recognized that the immune system presents a dual role in cancer: it may inhibit the growth of malignancies, by suppressing proliferation of neoplastic cells or by destroying them, and it may promote a microenvironment facilitating the establishment and the persistence of cancer by selecting clones of malignant cells that are more likely to survive in an immunocomponent host. Several immunological mechanisms seem to play a crucial role in the control and suppression of
tumor development. Different subclasses of immune cells, including in particular cytotoxic CD8 T+ lymphocytes (CTLs), CD4 T cells with helper functions and natural killer cells, are involved in the immune response against tumor and their activity is subject to a fine and tight regulation.\(^{75,76}\) The tumors are often characterized by infiltrates of immune cells with an inflammatory phenotype.\(^{77,78}\) The accumulation of these cells is the result of an interaction between the immune system and the tumor during its development and growth.\(^{79}\) The size and the composition of these infiltrates vary considerably according to the type, grade and stage of the malignancies, and their presence confirms that the host immune system develops a response against tumor. This process is called immune surveillance.\(^{80}\) Although in the past some clinical studies showed that survival of patients with different kinds of cancer is improved by the presence of cancer-infiltrating cells,\(^ {81,82}\) in recent years, available research reports that the type, density, and location of immune cells within neoplastic tissue predicts clinical outcome and prognosis.\(^ {83-85}\)

In particular, it has been suggested that the presence of intratumoral T lymphocytes with an effector phenotype has a favorable impact on the prognosis of patients with hepatic,\(^ {86,87}\) colorectal\(^ {88,89}\) and ovarian carcinoma.\(^ {90}\) Although it is well-known that many malignancies present modified antigens able to stimulate cytotoxic T-cell responses, immune system activity is generally weak and unable to block neoplastic proliferation.\(^ {91}\) This situation is characterized by the ability of the cancer microenvironment to inactivate or decrease the functionality of effector cells of the immune system with anti-tumor properties and to induce a condition of immune tolerance.

Several mechanisms have been proposed to explain the reduced response of the immune system against cancer: partial antigenic masking, deregulation of antigenic processing events, inadequate co-stimulation or direct suppression of effector cells.\(^ {92}\) Therefore, a dynamic and self-maintaining interplay is generated through which immune cells affect the neoplastic microenvironment and are, in turn, influenced by it.\(^ {93}\) The final effect of this process is represented by the progressive weakening of the protective immune response and by the associated increase in cancer growth and progression.

In recent years, in the heterogeneous population of CD4+ T cells, a subset of specialized CD4+/CD25+ T lymphocytes with constitutive or induced regulatory immunosuppressive function (Tregs) has been identified and characterized. These T cells control the function of CD4+ and CD8+ effector cells, which exert cytotoxic and helper activities during the protective phase of immune response, modulating their proliferation and cytokine release. Therefore, Treg cells, that help to prevent the development of organ-specific autoimmune diseases, play an essential role in the preservation and in the maintenance of host homeostasis and self-tolerance.\(^ {88,89}\)

Several studies showed that Treg cell dysfunction is involved in the pathogenesis of several diseases, such as inflammatory bowel diseases, autoimmune hepatitis, HBV- and HCV-related chronic hepatitis, thyroiditis and, probably, malignancies. The population of Treg cells is heterogeneous and it includes lymphocytes with extremely intricate patterns of cellular surface receptors and of cytokine production. Briefly, according to experimental evidence, Treg lymphocytes may be subdivided into two categories: induced (iTreg) or natural (nTreg) T cells. The latter population represents approximately 5-10% of CD4+ subsets. It originates in the thymus and is characterized by constitutive expression of a CD4 CD25\(^{\text{high}}\) Foxp3 positive pattern and by suppressive properties against CD4+ and CD8+ T-effector lymphocytes.\(^ {34,95}\) It has been reported that Foxp3 transcription factor modulates development and differentiation of Treg cells,\(^ {96,97}\) therefore, it is considered a specific marker of this population. On the other hand, iTreg subsets are able to proliferate in peripheral tissues in response to antigenic stimulation and they may originate from circulating naïve CD4+ T lymphocytes in the presence of appropriate stimuli, such as entity, duration and modality of antigenic presentation, as well as type of microenvironment, where these cells and antigens engage. The activities of some interleukins, including IL-2, IL-10 and transforming growth factor (TGF-β) seems to promote a Treg phenotype in CD4+ CD25+ Foxp3+ naïve T populations.\(^ {98,99}\) Both Treg cells and several subsets of T lymphocytes with effector properties share CD4 and CD 25 receptors on their cellular surface and this makes it difficult to distinguish these different populations. Furthermore, other receptors such as glucocorticoid-induced tumor necrosis receptor and intracellular antigen-4 associated with cytotoxic T lymphocytes were proved not to be useful markers to identify Treg cells.

Fox3p itself may be transiently expressed on cellular surface of some effector T subsets as a result of their activation, and its intracellular localization makes it very hard to recognize Treg populations.\(^ {100-102}\) Therefore, some studies by several authors have attempted to identify specific receptor patterns, and to reliably characterize effector and regulatory T cells. Recently, CD 127, representing the \(\alpha\)-chain for IL-7 receptor, has been proposed as a very useful marker to distinguish these different populations. According to available evidence, Treg cells seem to express low titers of CD127 (Treg127low), whereas it has been reported that T lymphocytes with protective activities against pathogens or malignant cells exhibit high levels of this
marker (T CD127^{high}). However, elevated values of CD127 are detectable only on T-effector cells with a memory phenotype after engagement with specific antigen and the subsequent differentiation process.

Prevalence of Treg cells is increased in peripheral blood of patients with different types of neoplasias, including liver, colorectal, breast, esophageal, and gastric carcinoma compared to healthy subjects. An early enhancement of Treg subsets occurs also in neoplastic tissue, as reported in pre-malignant lesions and the percentage of Treg populations increases, depending on cancer stage and presence of metastases. The detection of Treg cells infiltrating regional lymph nodes adjacent to tumor also predicts an unfavorable outcome, as suggested by a study performed in women with ovarian carcinoma. Even in patients with pancreatic cancer, a specific interaction between host neoplastic and immune cells has been observed. In particular, it has been reported that Treg subsets exert strong immune-suppressive activities against CD8+ cytotoxic as well as CD4+ helper lymphocytes, possessing specific anti-cancer function.

Ikemoto showed a significant enhancement of CD4+Foxp3+ T-cell population in peripheral blood of patients with pancreatic carcinoma in comparison with healthy donors and that percentage of Treg subsets correlates with TNM stage. On the other hand, Hirao reported that the prevalence of CD4+Foxp3+ T cells, infiltrating pancreatic cancer tissue and regional lymph nodes, increases during the progression of this tumor, ranging from precursor lesions, such as PanINs and intraductal-papillary mucinous neoplasia, to invasive carcinoma. A high prevalence of these Treg subsets predicts a poor prognosis. Hinz suggested a novel mechanism of immune evasion in pancreatic carcinoma. To date, ectopic expression of Foxp3 has been detected in neoplastic tissue collected from patients suffering from this type of malignancy. These results show that pancreatic cancer and Treg cells share pathogenetic mechanisms. Therefore, neoplastic cells may mimic the function of Treg subsets, suppressing immune system activity and promoting tumor growth.

In infection/inflammation/cancer models, a preferential migration of Treg cells into pathological areas is regulated and modulated by expression of specific receptors and chemokines.

Several important homing receptors have been identified, such as chemokine receptors 4, 5, 6, 7 and 8 (CCR4, CCR5, CCR6, CCR7 and CCR8). They promote an efficient migration of Treg cells into sites where inflammatory or malignant processes are developing. It has been reported that, in murine and human cancers of pancreas, Treg lymphocytes detectable in neoplastic tissue exhibit CCR5 receptor and that tumor cells themselves produce ligands specific for CCR5, such as chemokine ligand 5 (CCL5). In a murine model, a reduced expression of CCL5 by pancreatic cancer cells reduces the ability of Treg lymphocytes to migrate into cancer tissues and, as a consequence, it induces a slowdown in tumor growth.

Conclusions

Recent studies are improving our knowledge on the risk factors and pathogenetic mechanisms involved in PAC development. For the first time, epidemiological research has shown that infectious pathogens, such as viruses, may have a role in pancreatic carcinogenesis, although the small number of available reports, nearly all performed in Asian populations, limits their validity to these ethnic groups and their results should be interpreted with caution. Therefore, further studies focusing on populations of different geographical areas and involving larger series of patients are required to confirm this association. It should be considered that, in Asian countries such as China, HBsAg and HBcAb positivity is the most frequent serum pattern in HBV-infected patients, whereas in Western nations, HBsAb positive/HBcAb positive or HBsAb negative/HBcAb positive antibody profiles are widespread in the population, because HBV mass vaccination has greatly reduced the incidence of HBsAg positivity. Unfortunately, it is not known if HBsAb positive/HBcAb positive or HBsAb negative/HBcAb positive status are associated with an increased probability of pancreatic cancer. To date, only three studies have evaluated the risk of development of pancreatic carcinoma in these subjects and two of these suggested a higher incidence of pancreatic cancer in individuals with HBsAg negative/HBcAb positive/HBsAb negative pattern in comparison with controls. Therefore, this antibody profile, which is generally considered to be a sign of previous and recovered HBV infection, might have clinical importance. So far, no definitive conclusions may be drawn. However, should a causal role of HBV/HCV in the promotion of pancreatic carcinogenesis be definitively confirmed in further well-designed and well-adjusted studies, screening programs might be justified in patients with active or previous hepatitis B and C viral infection, although for the moment these programs are not feasible given the methods currently available and their high costs. Furthermore, in recent years, our understanding of PAC pathogenesis, such as processes of cytogenesis and histogenesis, immune system activities against this cancer, as well as the dynamic network and cooperative interactions occurring in the pancreatic microenvironment among cancer, immune, endothelial, pancreatic stellate cells and modulating factors has considerably
increased. Unfortunately, for the moment, in spite of these apparently encouraging results, the prognosis of this malignancy is still very poor and its treatment has not substantially improved. Therefore, additional efforts are needed to increase our knowledge of the molecular events as well as of macroscopic and microscopic tissue modifications, detectable during initiation and progression of pancreatic carcinoma. The development of models that describe the possible steps involved in the early phases of cancer presentation, as well as in its progression, might be a very useful approach to effectively deal with this pathology. In particular, the tensegrity model, proposed by Donald Ingber, has provided convincing hypotheses on possible mechanisms occurring during the general process of carcinogenesis. Therefore, it might be time to take a step forward and to apply the principles of this experimental model to clinical practice. A similar approach might considerably improve our understanding of the pathogenetic mechanisms involved not only in carcinogenesis of pancreas, but also of other organs. This new study and investigation approach might provide an important advance in the diagnosis and treatment of PAC, and it might have a considerable impact on the comprehensive management of patients affected by this aggressive malignancy.

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