

A systematic review on pancreatic cancer stem cells as a novel paradigm for curing pancreatic cancer

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Abstract- Pancreatic cancer remains one of the deadliest cancers, which is usually diagnosed in an advanced state for which there are little or no effective therapies. In this perspective, efforts have been made to identify specific subset of cells that have the capability of producing differentiated progeny of cells. There are three major types of cancer stem cells (CSCs), but the most important type of CSCs nowadays are chemo-resistant and radio-resistant CSCs that are resistant from both - chemotherapy and radiotherapy. Hence, these chemo-resistant and radio-resistant CSCs were identified, which may provide some novel therapeutic approach to treat pancreatic cancer. Increased numbers of CD44⁺CD24⁺ESA⁺ cells were needed to generate tumors when injected into the pancreas. Identified CD44⁺CD24⁺ESA⁺ pancreatic CSCs showed the stem cell properties i.e. self-renewal, the ability to produce differentiated progeny. Several signaling pathways are upregulated in the pancreatic CSCs. Sonic Hedgehog (SHH) signaling molecule expression was very high in CD44⁺CD24⁺ESA⁺ cells compared to other normal pancreatic epithelial cells. Cyclopamine, a steroidal alkaloid, which has both teratogenic and anti-tumor activities, inhibited Hh signaling thereby inhibited pancreatic cancer growth *in vitro* and *in vivo*, suggesting that this signaling pathway has an early and critical role in the development of pancreatic cancer. Inhibition of Hh signaling by cyclopamine indicates that in the near future cyclopamine can be used as a drug to show some promising effect in the treatment of pancreatic cancer.

Index Terms- pancreatic cancer, adenocarcinoma, sonic hedgehog, stem cell, stem cell markers

I. INTRODUCTION

Pancreatic cancer is the fourth most frequent cause of cancer-related deaths; it also represents one of the most aggressive cancer types, with a high incidence of distant metastasis and mortality [1]. The detection of pancreatic cancer at early stage and/or the response to therapy are the major challenges in improving the clinical outcome of pancreatic ductal adenocarcinoma (PDAC) [2]. The main issue against successful therapy is represented by the absence of early diagnostic and prognostic markers as well as the unresponsiveness to radiation and chemotherapies [3]. Cancer stem cells (CSCs) appear to have a major role among other factors that contribute to the lack of success in the therapy of pancreatic malignancies. Cancer is characterized by cellular heterogeneity; CSCs, which represent a distinct subpopulation of cells, seem to be responsible for tumor initiation and persistency due to their properties of self-renewal [4] and multilineage differentiation. CSCs are considered as best candidates responsible for tumorigenesis, metastasis, and chemo- and radio-resistance as showed in the Figure 1 [5-7]. Understanding and properly addressing the challenge represented by CSCs appears as a logical, yet difficult task in anti-cancer strategies.

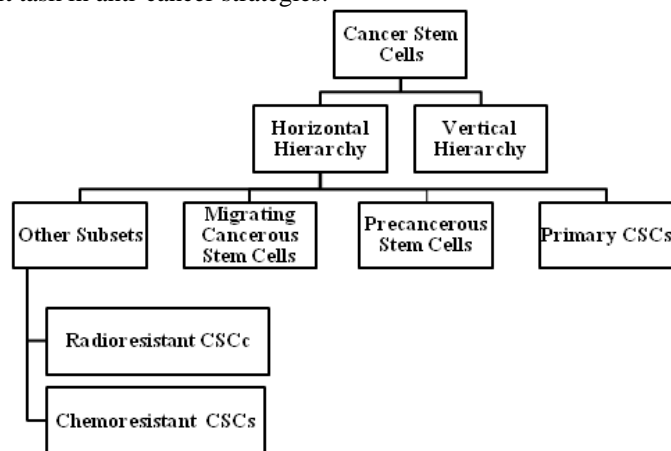


Figure 1: The schematic diagram shows various hierarchical populations of CSCs (After Liu et al. 2011).

II. PANCREATIC CSC AND THEIR SUBSETS

Emerging evidence suggests that the capability of a tumor to grow and propagate is dependent on a small subset of cells within a tumor, termed CSCs [8]. Although data have been provided to support this theory in human blood, brain and breast cancers, the identity of pancreatic CSCs has not been determined. After using a xenograft model in which primary human pancreatic adenocarcinomas were grown in immunocompromised mice, it was found that a highly tumorigenic subpopulation of pancreatic cancer cells expressing the cell surface markers CD44, CD24, and epithelial-specific antigen (ESA) [8]. Pancreatic cancer cells with the CD44⁺CD24⁺ESA⁺ phenotype (0.2–0.8% of pancreatic cancer cells) had a 100-fold increased tumorigenic potential compared with non-tumorigenic cancer cells, with 50% of animals injected with as few as 100 CD44⁺CD24⁺ESA⁺ cells forming tumors that were histologically indistinguishable from the human tumors from which they originated [8]. The enhanced ability of CD44⁺CD24⁺ESA⁺ pancreatic cancer cells to form tumors was confirmed in an orthotopic pancreatic tail injection model [8]. The CD44⁺CD24⁺ESA⁺ pancreatic cancer cells showed the stem cell properties of self-renewal, the ability to produce differentiated progeny, and increased expression of the developmental signaling molecule SHH. Identification of pancreatic CSCs and further elucidation of the signaling pathways that regulate their growth and survival may provide novel therapeutic approaches to treat pancreatic cancer, which is notoriously resistant to standard chemo- and radio-therapy [8].

III. CELL SURFACE MARKERS RELATED TO PANCREATIC CSC

A subpopulation of highly tumorigenic cancer cells within human pancreatic adenocarcinomas was identified using a xenograft model in which primary human pancreatic adenocarcinoma cells were implanted in immunocompromised mice. These cells displayed both self-renewal ability and ability to generate differentiated progeny, the ability to differentiate to recapitulate the phenotype of the tumor from which they were derived, and activation of developmental signaling pathways which are among the several features typically seen in stem cells. It was found that CD44, CD24, and ESA expressing cells represented the most highly tumorigenic population of pancreatic cancer cells compared with non-tumorigenic cells. After injection of only 100 CD44⁺CD24⁺ESA⁺ cells, resulting in tumor formation in 6 out of 12 of animals, indicating a 100 fold enhanced tumorigenic potential [8]. There was a remarkable similarity between the patients' primary tumors with that of the tumors derived from the pancreatic cancer cells. When 500 CD44⁺CD24⁺ESA⁺ cells were injected into a NOD/SCID (Non-obese diabetic/severe combined immune-deficient) mouse model, they showed development of tumor and H&E staining of the tumor (developed in the NOD/SCID mouse) exhibited phenotypically indistinguishable epithelial cancer cells when compared to H&E stained epithelial cells of the patient's primary tumor (Figure 2). On the contrary, no such tumor formation was observed after 500 CD44⁻CD24⁻ESA⁻ cells injection in a mouse model. [8].

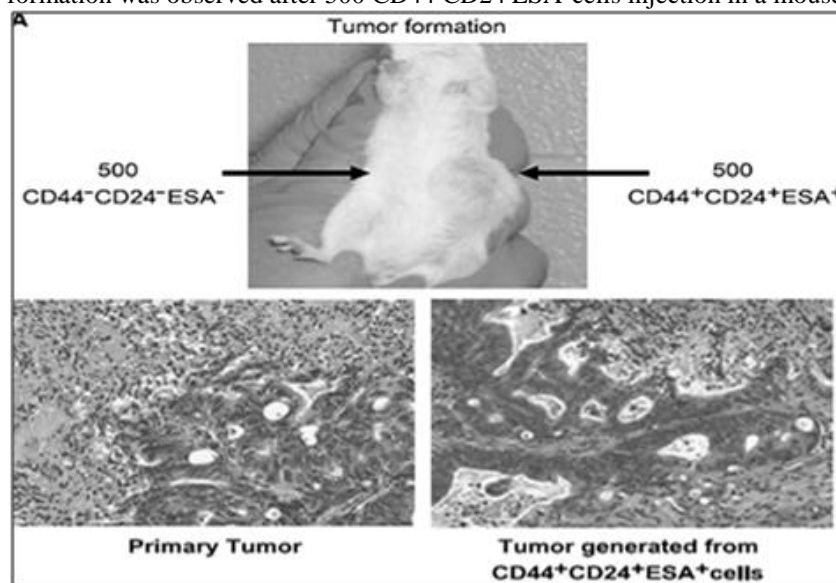


Figure 2: Tumor formation occurred at the injection site of 500 CD44⁺CD24⁺ESA⁺ cells and no tumor was formed at the injection site of 500 CD44⁻CD24⁻ESA⁻ cells (After Chenwei et al. 2007).

IV. PANCREATIC CANCER

Pancreatic cancer is an aggressive malignancy with one of the worst outcomes among all cancers. It is the fourth leading cause of cancer death in the United States with less than 5% five-year survival rate. The lifetime risk of developing pancreatic cancer in both men and women is about 1 in 79 i.e. 1.27% [9]. The American Cancer Society (ACS) estimated that new cases of 42,470 Americans (21,050 men and 21,420 women) would be diagnosed with pancreatic cancer during 2009. The ACS also estimated that 35,240 Americans (18,030 men and 17,210 women) would die of pancreatic cancer in 2009 [10]. Despite advances in molecular pathogenesis, problems such as drug resistance and susceptibility for metastasis make pancreatic cancer a major unsolved health problem in the

United States [11]. Unfortunately, pancreatic cancer is a rapidly invasive, metastatic tumor that is resistant to standard therapies [12,13].

At present, single agent based chemotherapy (e.g. Gemcitabine) is the key treatment for metastatic pancreatic adenocarcinoma. Recent data indicate that in addition to Gemcitabine, 5-FU plus a platinum agent such as oxaliplatin could be used as a therapeutic paradigm for early stage cancer patients [14]. However, none of the available current chemotherapeutic agents have objective response rates of over 10% [15]. The magnitude of this problem mandates the need for novel therapeutic agents. Recently, CSCs and epithelial-mesenchymal transition (EMT) type cells, which share molecular characteristics with CSCs, have been postulated to play critical roles in drug resistance and cancer metastasis in pancreatic cancer [16,17]. Recent studies suggest that CD44+CD24+ESA+ and ALDH1 could potentially be pancreatic CSC markers [5,18]. In addition, it is found that the recently identified intestinal stem cell marker DCAMKL-1 is also expressed in a small proportion of cells in the pancreas and in pancreatic CSC marker [19]. However, the role of DCAMKL-1 as a bona fide stem maker remains to be elucidated.

Pancreatic adenocarcinoma is a highly lethal disease, which is usually diagnosed in an advanced state. There are very few of effective therapies. It has the worst prognosis of any major malignancy (3% 5-year survival). It is the fourth most common cause of cancer death in the United States, with an annual death rate of 31,000 people per year [20]. Although there are many advances in surgical and medical therapy, but it has less effect on the mortality rate of this disease. Identification of pancreatic CSCs and then exposition of the signaling pathways regulating pancreatic cancer cell's growth and survival may provide an interesting therapeutic approach to treat pancreatic cancer [8].

V. ROLE OF DIFFERENT SIGNALING PATHWAYS IN PANCREATIC CANCER

Although in normal stem cells the self-renewal pathways are tightly controlled but in tumor initiating cells they may be activated constitutively. They may have an improper regulation through some epigenetic or genetic changes which ultimately lead to uncontrolled growth. Several studies reveal that during pancreatic cancer many signaling pathways are activated that ultimately regulate the uncontrolled self-renewal and proliferation of different CSCs. There are some specific signaling pathways that play a major role in the self-renewal of CSCs such as Wnt, Notch and Hedgehog (Hh) signaling pathways [21,22]. It has been found that CSCs in pancreatic cancer are involved in the epithelial to mesenchymal transition (EMT). This process involves phenotypic changes of epithelial cells towards mesenchymal cells. Although EMT is usually activated during embryogenesis by the signaling pathways, many EMT-activating transcriptional signals are up regulated (Figure 3) [23].

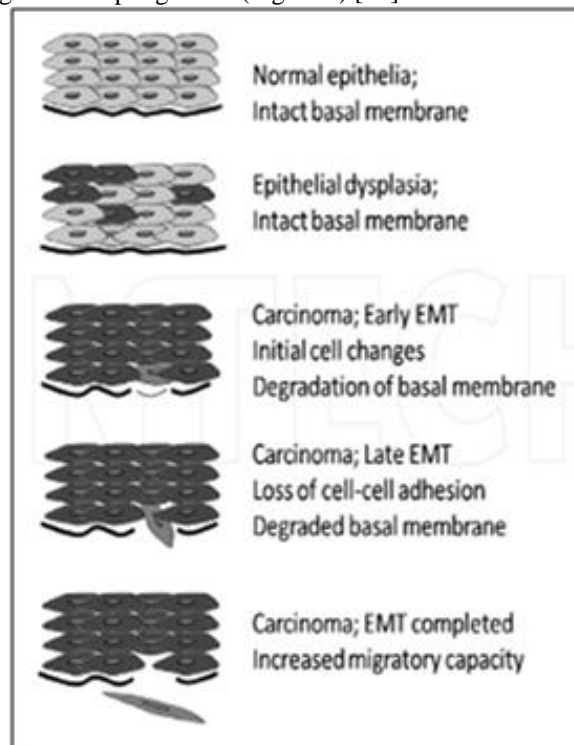


Figure 3: Epithelial-to-mesenchymal transition process (After Cristiana et al. 2004).

A. Wnt signaling pathway

It was found that nuclear β -catenin is necessary for EMT. *In vitro* and *in vivo* studies shows that when activated β -catenin increase self-renewal capacity resulting into development of epithelial cancer in mice [24]. Wnt blocks glycogen synthase kinase 3 (GSK-3 β)

activity thus stabilizes Snail and β -catenin levels. This process is related to cancer metastasis and is thus involved in the EMT programme. Also Snail interacts with β -catenin and enhances Wnt signaling.

B. Notch signaling pathway

Recent studies reveal that Notch signaling activates EMT in cancer because this signaling pathway is responsible for cell fate, proliferation, differentiation, apoptosis and the maintenance of stem cells. It is thought that Notch can regulate endothelial and mesenchymal markers to sustain mesenchymal transformation. Notch pathway increases cellular migration by activating nuclear factor kappa β (NF- κ B), Matrix metalloproteinase 9 and vascular endothelial growth factor (VEGF) in pancreatic cancer cells. Several studies suggest that Notch inhibition can reverse EMT in the mesenchymal to epithelial transition (MET). This phenomenon is considered to be a promising therapeutic strategy in cancer treatment [25].

C. Hedgehog signaling

Hedgehog signaling is a self-renewal pathway. It is involved in embryonic cell growth and organogenesis. Hh is also involved in regulating genes associated with cell proliferation, differentiation, and cell motility. Hh pathway is normally inactive in adult organs but become very active in cancer. In such cases Hh increases myofibroblast differentiation and production of extracellular matrix which in turn enables the EMT process in cancer cells. The EMT process is actively involved in the generation of tumor metastasis and tumor recurrence. Such CSCs that have undergone EMT display resistance to therapy are also generated during Hh signaling. Cellular migratory potential is also increased by up-regulation of Mucin-4 (MUC4) and fibroblast growth factor receptor 1 (FGFR-1) stabilization. Other studies show that the process in pancreatic cancer can also be regulated by Forkhead box protein M1 (FoxM1)-caveolin, GLI-Kruppel family member (GLI1), hepatocyte growth factor (HGF) or platelet-derived growth factor (PDGF). Hence it can be said that EMT type pancreatic tumor cells represent a highly important research focus for the therapies aiming at reducing or preventing invasion, metastasis and therapeutic resistance in pancreatic cancer. In vertebrates, there are basically three homologs of Hh sonic Hh, desert Hh and Indian Hh, which are expressed at different stages of development indicating that they may have different functions. Mutation in SHH pathway results into increased cellular proliferation, transformation ultimately leading to cancer. It has been found that inhibition of SHH signaling reduces metastatic tumor formation in pancreatic adenocarcinoma and prostate cancer [26]. Recently, it was found that the level of SHH was very high in pancreatic CSCs indicating that SHH play a key role in adult stem cell renewal. In highly tumorigenic pancreatic cancer cell population, there was an increase in the expression of developmental signaling molecule SHH. SHH is a signaling molecule which is highly expressed in the developing embryos. RT-PCR of three samples of normal pancreas and three separate pancreatic cancer xenografts showed that SHH expression was highest in CD44⁺CD24⁺ESA⁺ cells, which suggests that SHH expression is markedly upregulated in pancreatic CSCs as shown in the Figure 4 [8].

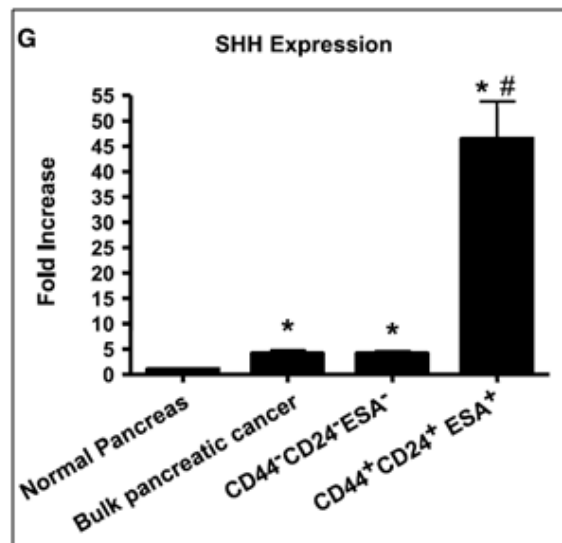


Figure 4: mRNA expression of SHH, important in developmental signaling pathways, in normal pancreas, bulk pancreatic cancer cells, nontumorigenic CD44⁺CD24⁺ESA⁻ pancreatic cancer cells and highly tumorigenic CD44⁺CD24⁺ESA⁺ pancreatic cancer cells (After Chenwei et al. 2007).

VI. ACTIVATION OF HEDGEHOG SIGNALING PATHWAY IN PANCREATIC CANCER

Different important molecules that are present in Hh signaling pathway were detected after performing immune-histochemical studies of pancreatic cancer tumors of human patient and of NOD/SCID mice. Patched 1 (Ptc 1) receptor and smoothed protein were detected in tumors derived from pancreatic cancer (Figure 5) [27].

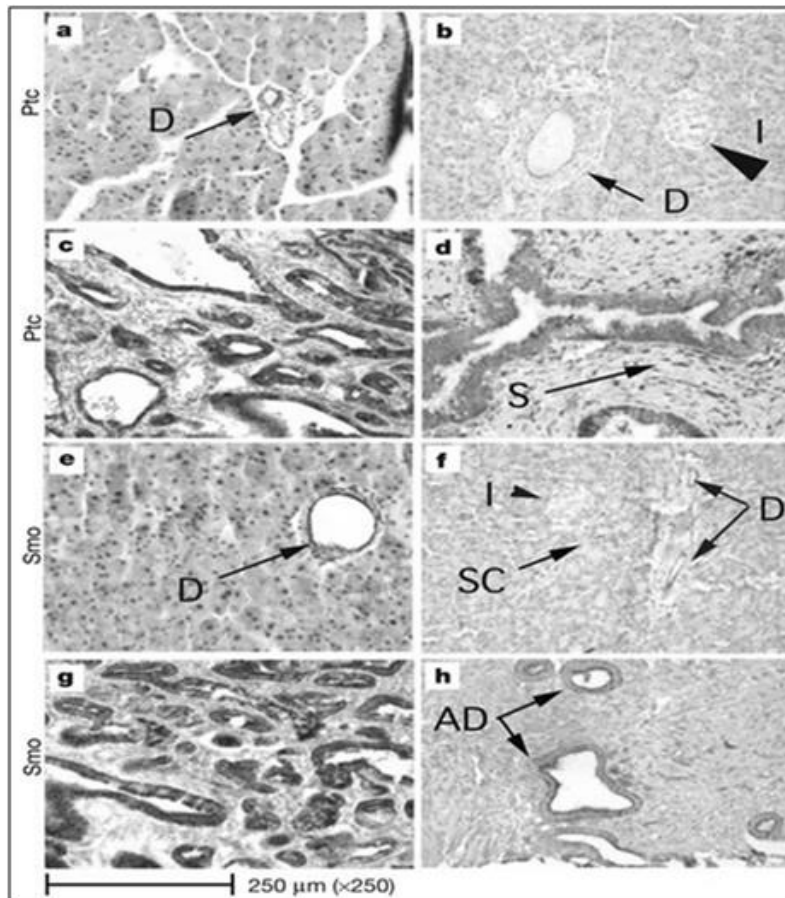


Figure 5: Expression of different Hh signaling molecules in control and pancreatic cancer in human and mice. No expression of Ptc 1 receptor in A) mouse, B) human pancreas; C) Overexpression of Ptc 1 in PdxSHH mice [where there is a misexpression of SHH in the pancreatic endoderm] and in D) neoplastic human pancreas; E-H) Overexpression of Ptc 1 throughout reactive stroma surrounding the epithelium (S) ; No expression of Smoothened [Smo] in E) normal duct epithelium [D] of control mouse or in ; F) human pancreas; Smo is detected in the abnormal pancreatic epithelium of G) Pdx-SHH mice and in ; H) abnormal pancreatic epithelium (After Thayer et al. 2003).

VII. ROLE OF CYCLOPAMINE IN HEDGEHOG SIGNALING PATHWAY

Cyclopamine is a steroidal alkaloid or phytochemical, found in the plant *Veratrum californicum*, commonly called the corn lily. It interacts directly with Smo and thus inhibits Hh signaling [28-30]. It was found that 7 days after the administration of cyclopamine, there was no change in the cell density or morphology in control pancreatic cells; but there was a marked reduction in the cell density of the cancer cell lines. Those cells detached from the culture plates during the treatment period. The alkaloid did not induce apoptosis but it marginally reduced the proliferation of Smo cyclopamine responsive cell lines. Cyclopamine was used to test whether pancreatic adenocarcinoma require Hg signaling for their own proliferation and survival [27]. It was found that cyclopamine inhibits pancreatic cancer growth *in vitro* and *in vivo* by inhibiting Hh signaling. In pancreatic orthotopic xenograft models, a combination of cyclopamine and gemcitabine inhibited metastatic spread and primary tumor growths [31]. Hence, it suggests that this signaling pathway plays an early and important role in the development of pancreatic cancer.

VIII. DISCUSSION

In this review, the role of most important pancreatic CSCs in the development of pancreatic cancer with special focus on some developmental signaling pathways is summarized. Some highly tumorigenic cancer cells which expressed CD44⁺CD24⁺ESA⁺ cell surface markers are responsible for developing pancreatic cancer. These cells have the ability to self-renew and can produce differentiated progeny and also they can recapitulate the phenotype of the progenitor tumor. It was found that in human breast cancer patients the tumorigenic cancer cell population was CD44⁺CD24⁺ESA⁺. In human brain tumor and prostate cancer, expression of tumorigenic cancer cell population was CD133⁺ and they had a high tumorigenic potential [32-34]. The tumorigenic cancer cell population for melanoma was CD20⁺ cells. All these studies of different CSCs suggests that there are some common markers in different cancer cells but one unique phenotype for marker CD24⁺ is found only in case of pancreatic cancer. These stem cells can

also activate different developmental signaling pathways. It has been found that several developmental signaling pathways such as Wnt, Hh and Notch are activated during pancreatic adenocarcinoma. The mRNA expression of SHH level is very high in pancreatic CSCs. The alkaloid cyclopamine have successfully reduced the tumor growth *in vitro* in NOD/SCID mice models. Hh inhibitors such as cyclopamine may thus be used as a promising drug in the treatment of one of the most aggressive cancer - pancreatic cancer. Identification of pancreatic stem cells and identifying the role of Hh signaling pathway and explication of other signaling pathways which regulate their growth and survival may provide innovative therapeutic approaches in the treatment of pancreatic cancer.

IX. CONCLUSION

CD44⁺CD24⁺ESA⁺ pancreatic cancer cells showed stem cell properties of self-renewal, increased expression of the developmental signaling molecule SHH. CD44⁺CD24⁺ESA⁺ cells formed tumors, histologically indistinguishable from human pancreatic tumors from which they originated. Cyclopamine having teratogenic and antitumor activities inhibits pancreatic cancer growth *in vitro* and *in vivo* by blocking Hh signaling pathway. Identification of pancreatic CSCs, by understanding the signaling pathways that regulate their growth, survival may provide novel therapeutic approaches to treat pancreatic cancer as it is notoriously resistant to standard chemo and radiotherapy. Hh signaling inhibition by cyclopamine indicates that it may be used as a potent drug to show some promising effect in the treatment of pancreatic cancer in the near future.

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