Translational Research in Radiotherapy of Pancreatic Adenocarcinoma: A Review

Arcelli A[1], Cammelli S[1], Frakulli R[1], Farina E[1], Guido A[1], Cellini F[2], Macchia G[3], Mattiucci GC[4], Buwenge M[1], Morganti AG[1]

1   Radiation Oncology Center, Dept. of Experimental, Diagnostic and Specialty Medicine - DIMES, University of Bologna, Bologna, Italy
2   Radiotherapy Department, Campus Biomedico University, Rome, Italy
3   Radiotherapy Unit, Fondazione Giovanni Paolo II, Campobasso, Italy
4   Radiation Oncology Department, Catholic University of the Sacred Heart, Rome, Italy

Abstract

Cancer of the pancreas is one of the tumors with the worst prognosis. Radiation therapy has been proposed as an exclusive or adjuvant/neoadjuvant treatment. However, its effectiveness is limited by the low tumor radiosensitivity and by the frequent and early hematogenous metastasis. In order to improve the results of radiotherapy, various protocols of concurrent chemoradiation have been extensively tested. The clinical results have been disappointing and the role of concurrent radio-chemotherapy combination is widely debated.

More recently, a series of preclinical studies attempted to identify potential targets in pancreatic cancer to allow individualized treatments, to increase radiosensitivity and, more generally, to improve effectiveness of radiation therapy. In this context, EGFR, KRAS, p53, and ATDC have been studied as potential targets. While cetuximab, nelfinavir, recombinant adenovirus-p53 and cyclopamine were tested as potential targeted therapies.

Although most of published studies are preliminary (phase I or phase II), they produced some promising results and therefore larger phase III trial can be justified. It is possible, in the future, that the standard management of pancreatic carcinoma will be represented by multi-modality treatments combining conventional therapeutic strategies (chemotherapy, chemo-radiation) with novel targeted drugs.

Key words: Radiotherapy; Translational research; Pancreatic neoplasms; Review

Corresponding author:
Alessandra Arcelli MD

✉ alearceese@hotmail.it

UO Radioterapia, Ospedale S.Orsola-Malpighi, Università di Bologna, via Giuseppe Massarenti 9, 40138, Bologna, Italy

Tel: +39-051-348848

Fax: +39-051-6364336

Introduction

The prognosis of pancreatic adenocarcinoma (PAC) is so unfavorable that the annual incidence and mortality rates of this disease are equivalent [1]. PAC is currently the fourth leading cause of cancer mortality in the United States but in 2030 forecasts it represents the second leading cause of cancer mortality with an extremely low 5-year overall survival [2,3]. Particularly, in 2013 over 45,000 new cases of PAC were diagnosed in the United States [2] and of these only 20% presented with resectable disease [4-7].

Actually the only chance of cure in PAC is radical surgery with tumor free margins [5,8,9]. However, only in a small percentage (20%) of highly selected patients is a complete surgical resection feasibile [10]. In fact, about 40% of patients with PAC present at diagnosis with inoperable locally advanced disease [11]. Furthermore, even in patients undergoing radical surgery, 5-year

survival remains about 15-20% with 30% mortality rate due to local recurrence [11].

Although the cure rates of PAC are marginally improved by the use of adjuvant therapies, definition of a standard approach for the treatment of locally advanced disease is currently not available [12]. The international guidelines suggest a multidisciplinary approach based on different multimodal strategies (chemotherapy alone or chemoradiation or both) [13,14]. An emerging trend is represented by the possibility to adapt the treatment based on patients characteristics (performance status, age, comorbidities) [14] and tumor characteristics (stage, grading and genetic profile of their disease) [15]. Anyway, despite recent advances, the prognosis of patients affected by PAC is dismal, highlighting the need for new therapeutic approach based on preclinical and translational studies.

In the last decades progress in the research on PAC biology have

led to cancer microenvironment becoming a target for therapies [12,16]. In fact, tumor proliferation can be modulated by the stromal compartment regulating blood supply and hypoxia with the potential of enhancing the chemotherapy delivery to the tumor.

The principal cause of treatment failure in PAC is the intrinsic chemoresistance and radioresistance [17]. This feature is mostly due to the presence of tumor hypoxic regions significantly correlated with worst prognosis. Several mechanism (apoptosis inhibition, angiogenesis stimulation, up-regulation of genetic and epigenetic changes) can increase hypoxia hence increasing even tumor invasion and resistance to treatments [18]. With the aim to overcome tumor resistance to standard treatments, some new biological therapies are targeting the stroma [19-24], the Epidermal Growth Factor Receptor (EGFR) [25] or the Vascular Endothelial Growth Factor Receptor (VEGFR).

The biology of PAC having gained a better understanding, translational research is now directed towards identification of novel and effective agents in curative and adjuvant therapies [12,26]. On the basis of these premises, the aim of this review is to briefly summarize the main evidences in the field of translational research in radiotherapy treatments of PAC.

Preclinical and Clinical Studies

The EGFR is overespressed in over 90% of pancreatic cancer samples [27,28]. Based on this high rate, several studies were carried out to evaluate the possibility of selectively targeting this receptor. A phase II trial enrolled 21 patients with advanced PAC receiving a cetuximab (a chimeric monoclonal antibody inhibiting the EGFR) loading dose (400 mg/m2) and a weekly cetuximab dose (250 mg/m2) during RT (50.4 Gy in 28 fractions) was published [25]. An ancillary feasibility study of on-trial patient blood and skin sampling was also performed. The results reported were grade ≤ 2: acute toxicity: 70%, median overall survival: 7.5 months, 1-year actuarial survival: 33% and 3-year overall survival: 11%. The authors concluded that cetuximab favors PAC radiosensitization by achieving better tumor control with marginal toxicity. Before this study, in 2005 [29] and 2011 [30], other authors demonstrated the stimulating effect of EGFR in repairing radiation-induced DNA damage. Patient skin and blood samples yielded sufficient RNA and proteins, which encouraged new studies on future biomarker analysis with the aim to predict individual tumor response [25].

Some studies in PAC demonstrated that EGFR signaling is required for KRAS driven pancreatic tumorigenesis [31,32] and that KRAS is widely mutated in PAC (80-100% of cases) [33,34]. In 2009 an American group [35] prospectively evaluated plasma KRAS as a potential marker of response in 12 patients with locally advanced pancreatic cancer receiving induction gefitinib (an EGFR tyrosine kinase inhibitor) and chemoradiotherapy (paclitaxel concurrent to radiotherapy). KRAS codon 12 mutations confirmed by direct sequencing were detected using a two-stage restriction fragment length polymorphism-polymerase chain reaction assay on patients' plasma both before and after therapy. Five patients presented KRAS mutations pretreatment. In 3 of those KRAS

was undetectable post-treatment and these patients showed prolonged overall survival (8, 11 and 21 months) compared to patients who retained KRAS after treatment (2 and 4 months). Therefore, changes in KRAS plasma level may predict response to treatment. A trial based on KRAS targeting with an inhibitor failed to show clinical results [36], probably because no available effective targeting of KRAS is possible due to its intrinsic mode of action based on current knowledge.

The efficacy of radiotherapy in PAC is limited by the low radiation doses generally used, due to the risk of toxicity particularly in patients treated with concurrent chemoradiation [25]. Therefore, some studies were directed towards the association of molecular agents with radiotherapy to intensify response and minimize toxicity. In 2008 [37] a group from Oxford conducted a phase I trial on the association of nelfinavir to chemoradiotherapy in 12 patients with borderline resectable or unresectable PAC. The rational of this study was the mediation role of PI3 kinase for EGFR and KRAS from preclinical studies. Nelfinavir is a protease inhibitor for PI3 kinase that potentially may have radiosensitizing effects [38-40]. In 5 of 10 patients who completed chemoradiotherapy the authors observed partial CT responses, with 5 complete PET responses and 6 R0 resection. The median survival was 18 months with an acceptable non haematological toxicity. Now a Cancer Research UK group (Clinicaltrials.gov NCT02024009) is conducting a phase II clinical randomized trial (SCALOP 2) on nelfinavir which started in April 2015. Patients are treated with induction chemotherapy (gemcitabine and nab-paclitaxel) and those obtaining a stable disease will be randomized to receive the same regimen of induction chemotherapy, conventional chemoradiotherapy (capecitabine with radiotherapy at low doses), high doses chemoradiotherapy (capecitabine with radiotherapy at high doses), low doses chemoradiotherapy with nelfinavir or high doses chemoradiotherapy with nelfinavir.

In over 75% of the pancreatic carcinoma p53 oncosuppressor, promoting DNA repairing from radiation damage, is mutated thus increasing tumor radioresistence [41,42]. In 2011 a Chinese group assessed the efficacy of the combination of chemoradiotherapy (Intensity Modulated Radiotherapy plus gemcitabine) and recombinant adenovirus-p53 (rAd-p53) in 15 patients with unresectable pancreatic carcinoma. In this pilot study, target therapy with rAd-p53 showed encouraging preliminary results in terms of survival and toxicity [43] (**Table 1**).

Furthermore, other strategies has been used to improve tumor radio-sensitivity by targeting different molecules. In 2013 a Chinese preclinical study analyzed cyclopamine, an hedgehog-inhibitor, as radiosensitizing agent on tumor pancreatic cells through a KRAS-indipendent pathway [44]. In 2014 an American group published the results of an in vitro and in vivo research on ATDC/TRIM29, a binding protein of DNA enhancing tumor growth [45]. It was found that ATDC is overexpressed in PAC, playing a role on DNA damage resulting in increased tumor radioresistence. More interestingly, both in vitro and in vivo assays, the knockdown of this protein sensitizes PAC to ionizing radiation.

Finally, another emerging and potentially useful strategy in combined modality treatment of PAC is represented by the use of nanomedicine techniques to optimize concurrent

Table 1 target therapy with rAd-p53 showed encouraging preliminary results in terms of survival and toxicity.

Author/ Year	Patients	Study design	Treatment	Main findings
Brunner TB et al., 2008 [37]	18 unresectable/ borderline resectable CAP	Phase I	RT and concurrent cisplatin + gemcitabine + nelfinavir	acceptable toxicity and promising activity: 5 PR at CT scan; 5 CR at PET; 2 SD; 6 RO resections with 1 complete pathological response.
Olsen CC et al., 2009 [35]	11 locally advanced CAP	Phase I	7-day induction gefitinib (250 mg) followed by daily gefitinib + concurrent CRT (50.4 Gy/28 fractions of RT + weekly paclitaxel, 40 mg/m2 IV) followed by gefitinib maintenance	daily gefitinib + concurrent CRT was reasonably tolerated. Survival times were favourable in patients with undetectable post-treatment k-ras mutations post-treatment.
Li JL et al., 2011 [43]	15 unresectable CAP	Phase I-II	rAd-p53 + CRT (IMRT 55-60 Gy/25-30 fx + gemcitabine 350 mg/mq weekly for 6 weeks)	rAd-p53 + CRT in unresectable CAP was well tolerated and showed encouraging clinical benefit response (53.3%) and disease control rate (80.0%). Median PFS and OS were 6.7 and 13.8 months, respectively.
Rembielak AI et al., 2014 [25]	21 locally advanced CAP	Phase II	cetuximab (loading dose: 400 mg/m2 + weekly dose: 250 mg/m2) + concurrent RT (50.4 Gy/28 fx)	cetuximab inhibits EGFR-mediated radioresistance but does not control metastatic progression (6 months post-treatment: 90% SD; only 33% free from metastatic progression). Median OS: 7.5 months. Extended survival was associated with high-grade acneiform rash (p=.003), post-treatment stable disease (p=.006) and pretreatment CA19.9 level (p= .004).

Abbreviations: CAP: Carcinoma of the Pancreas; CR: Complete Response; CRT: Chemo-Radiation; Fx: Fractions; OS: Overall Survival; PFS: Progression-free Survival; PR: Partial Response; rAd-p53: Recombinant Adenovirus-p53; RT: Radiotherapy; SD: Stable Disease.

chemoradiotherapy. In fact, nanoparticles may deliver chemotherapy with a preferential accumulation into the tumor and with a minimale uptake in normal tissue. This makes nanoparticles an ideal way to deliver chemotherapy in radiochemotherapy regimens [46].

Conclusions

Nowadays, PAC remains one of the hardest challenges for clinical and translational researches. Despite some improvement in prognosis, the cost in terms of life and medical expenditures are very high. Innovative treatments facing PAC using different treatment strategies could be the best chance to make PAC a largely curable disease [47]. Progresses in translational research have surely produced some promising results so that probably, in the future, the gold standard treatment of PAC will be represented by multi-modality treatments combining conventional therapeutic strategies (chemotherapy, radiochemotherapy) with novel targeted drugs.

Conflicts of interest notification

No actual or potential conflicts of interest do exist regarding this paper.

References

1. Rockahrt AC, Rothenberg ML, Berlin JD (2005) Treatment for pancreatic cancer: current therapy and continued progress. Gastroenterology 128: 1642–1654.

2. Siegel R, Miller KD, Jemal A (2015) Cancer statistics 2015. CA Cancer J Clin 65: 5-29.

3. Rahib L, Smith BD, Aizenberg R (2014) Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2913-2921.

4. Li D, Xie K, Wolff R (2004) Pancreatic cancer. Lancet 363: 1049-1057.

5. Gudjonsson B (1987) Cancer of the pancreas. 50 years of surgery. Cancer 60: 2284-2303.

6. Yeo CJ, Abrams RA, Grochow LB, Sohn TA, Ord SE, et al. (1987) Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative adjuvant chemoradiation improves survival. A prospective, single-institution experience. Ann Surg 225: 621–633.

7. Hidalgo M (2010) Pancreatic cancer. N Engl J Med 362: 1605–1617.

8. Wagner M, Redaelli C, Lietz M, Seiler CA, Friess H, et al. (2004) Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. Br J Surg 91: 586–594.

9. Gurusamy KS (2014) Resection versus other treatment for locally advanced pancreatic cancer. Cochrane Database Syst Rev 2: CD010244.

10. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, et al. (2005) Cancer statistics, 2005. CA Cancer J Clin 55: 10–30.

11. Hishinuma S, Ogata Y, Tomikawa M (2006) Patterns of recurrence after curative resection of pancreatic cancer, based on autopsy findings. J Gastrointest Surg 10: 511-518.

12. Huguet F, Mukherjee S, Javle M (2014) Locally advanced pancreatic cancer: the role of definitve chemoradiotherapy. Clin Oncol 26: 560-568.

13. NCCN guidelines. Pancreatic adenocarcinoma. Version 1.2015 (guidelines.https:// www.nccn.org /store /login /login. aspx?ReturnURL=http://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf).

14. Li J, Ng J, Allendorf J, Saif MW (2011) Locally advanced pancreatic adenocarcinoma: are we making progress? Highlights from the "2011 ASCO Annual Meeting". Chicago, IL, USA JOP 12: 347-350.

15. Halperin MD, Varadhachary GR (2014) Resectable, Borderline resectable, and locally advanced pancreatic cancer: what does it matter? Curr. Oncol Rep 16: 366.

16. Ahn DH, Ko AH, Meropol NJ, Bekaii-Saab TS (2015) Making sense of current and emerging therapies in pancreatic cancer: balancing benefit and value. Am Soc Clin Oncol Educ Book 35: 222-227.

17. Sarkar S, Azab B, Quinn BA, Shen X, Dent P, et al. (2014) Chemoprevention Gene Therapy (CGT): novel combinatorial approach for preventing and treating pancreatic cancer. Curr Mol Med 14: 125-140.

18. Sun JD, Liu Q, Ahluwalia D (2015) Efficacy and safety of the hypoxia-activated prodrug TH-302 in combination with gemcitabine and nab-paclitaxel in human tumor xenograft models of pancreatic cancer. Cancer Biol Ther 16: 438-449.

19. Chu GC, Kimmelman AC, Hezel AF (2011) Stromal biology of pancreatic cancer. J Cell Biochem 101: 887-907.

20. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674.

21. Hwang RF, Moore T, Arumugam T (2008) Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res 68: 918-926.

22. Vonlaufen A, Phillips PA, Xu Z (2008) Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. Cancer Res. 68: 7707-7710.

23. Ikenaga N, Ohuchida K, Mizumoto K (2010) CD10 pancreatic stellate cells enhance the progression of pancreatic cancer. Gastroenterology 139: 1041-1105.

24. Lunardi S, Muschel RJ, Brunner TB (2014) The stromal compartments in pancreatic cancer: are there any therapeutic targets? Cancer Lett 343: 147-155.

25. Rembielak AI, Jain P, Jackson AS (2014) Phase II Trial of Cetuximab and Conformal Radiotherapy Only in Locally Advanced Pancreatic Cancer with Concurrent Tissue Sampling Feasibility Study. Transl Oncol 7: 55-64.

26. Singh P, Srinivasan R, Wig JD (2011) Major molecular markers in pancreatic ductal adenocarcinoma and their roles in screening, diagnosis, prognosis, and treatment. Pancreas 40: 644-652.

27. Tobita K, Kijima H, Dowaki S, Kashiwagi H, Ohtani Y, et al. (2003) Epidermal growth factor receptor expression in human pancreatic cancer: significance for liver metastasis. Int J Mol Med 11: 305-309.

28. Fjällskog ML, Lejonklou MH, Oberg KE, Eriksson BK, Janson ET (2003) Expression of molecular targets for tyrosine kinase receptor antagonists in malignant endocrine pancreatic tumors. Clin Cancer Res 9: 1469-1473.

29. Dittmann K, Mayer C, Fehrenbacher B (2005) Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J Biol Chem 280: 31182–31189.

30. Dittmann K, Mayer C, Fehrenbacher B (2011) Nuclear epidermal growth factor receptor modulates cellular radiosensitivity by regulation of chromatin access. Radiother Oncol 99: 317–322.

31. Ardito CM, Grüner BM, Takeuchi KK (2012) EGF receptor is required for KRAS-induced pancreatic tumorigenesis. Cancer Cell 22: 304–317.

32. Navas C, Hernández PI, Schuhmacher AJ (2012) EGF receptor signaling is essential for K-Ras oncogenedriven pancreatic ductal adenocarcinoma. Cancer Cell 22: 318–330.

33. Almoguera C, Shibata D, Forrester K (1988) Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 53: 549-554.

34. Tada M, Omata M, Kawai S (1993) Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. Cancer Res 53: 2472-2474.

35. Olsen CC, Schefter TE, Chen H (2009)Results of a phase I trial of 12 patients with locally advanced pancreatic carcinoma combining gefitinib, paclitaxel, and 3-dimensional conformal radiation: report of toxicity and evaluation of circulating K-ras as a potential biomarker of response to therapy. Am J Clin Oncol 32: 115-121.

36. Macdonald JS, McCoy S, Whitehead RP (2005) A phase II study of farnesyl transferase inhibitor R11577 in pancreatic cancer: a

Southwest Oncology Group (SWOG 9924) study. Invest New Drugs 23: 485-487.

37 Brunner TB, Geiger M, Grabenbauer GG (2008) Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. J Clin Oncol 26: 2699-2706.

38 Pajonk F, Himmelsbach J, Riess K (2002) The human immunodeficiency virus (HIV)-1 protease inhibitor saquinavir inhibits proteasome function and causes apoptosis and radiosensitization in non-HIV-associated human cancer cells. Cancer Res 62: 5230-5235.

39 Gupta AK, Cerniglia GJ, Mick R (2005) HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both in vitro and in vivo. Cancer Res 65: 8256-8265.

40 Plastaras JP, Vapiwala N, Ahmed MS (2008) Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans. Cancer Biol Ther 7: 628-635.

41 Morran DC, Wu J, Jamieson NB (2014) Targeting mTOR dependency in pancreatic cancer. Gut 63: 1481-1489.

42 Fiorini C, Cordani M, Padroni C (2015) Mutant p53 stimulates chemoresistance of pancreatic adenocarcinoma cells to gemcitabine. Biochim Biophys Acta 1853: 89-100.

43 Li JL, Cai Y, Zhang SW (2011) Combination of recombinant Adenovirus-p53 with radiochemotherapy in unresectable pancreatic carcinoma. Chin J Cancer Res 23: 194-200.

44 Wu XY, Che J, Sun KK (2013) Cyclopamine increases the radiosensitivity of human pancreatic cancer cells by regulating the DNA repair signal pathway through an epidermal growth factor receptor–dependent pathway. Mol Med Rep 8: 979-983.

45 Wang L, Yang H, Palmbos PL (2014) ATDC/TRIM29 phosphorylation by ATM/MAPKAP kinase 2 mediates radioresistance in pancreatic cancer cells. Cancer Res 74: 1778-8847.

46 Werner ME, Foote MB, Wang AZ. Chemoradiotherapy of human tumors: novel approaches from nanomedicine. Curr Pharm Des 18: 2830-2837.

47 Teague A, Lim KH, Wang-Gillam A. Advanced pancreatic adenocarcinoma: a review of current treatment strategies and developing therapies. Ther Adv Med Oncol 7: 68-84.