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Original article

Polyisoprenylated methylated protein methyl esterase: A putative biomarker and therapeutic target for pancreatic cancer

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ABSTRACT

Pancreatic cancer is the most deadly neoplasm with a 5-year survival rate of less than 6%. Over 90% of cases harbor K-Ras mutations, which are the most challenging to treat due to lack of effective therapies. Here, we reveal that polyisoprenylated methylated protein methyl esterase (PMPMEase) is overexpressed in 93% of pancreatic ductal adenocarcinoma. We further present polyisoprenylated cysteinyl amide inhibitors (PCAIs) as novel compounds designed with structural elements for optimal *in vivo* activities and selective disruption of polyisoprenylation-mediated protein functions. The PCAIs inhibited PMPMEase with K_i values ranging from 3.7 to 20 μM. The 48 h EC_{50} values for pancreatic cancer Mia PaCa-2 and BxPC-3 cell lines were as low as 1.9 μM while salirasib and farnesylthiosalicylamide were ineffective at 20 μM. The PCAIs thus have the potential to serve as effective therapies for pancreatic and other cancers with hyperactive growth signaling pathways mediated by Ras and related G-proteins.

1. Introduction

Pancreatic ductal adenocarcinoma (PDA) accounts for approximately 95% of all pancreatic cancers (PC) and remains one of the deadliest neoplasms as the number of new diagnoses closely mirrors deaths. The poor prognosis is attributed to lack of early symptoms, with >50% of patients diagnosed at a late stage of the disease [1]. Epidermal growth factor receptor (EGFR) plays a vital role in the progression of PC [2] and is associated with poor prognosis [3]. Research to uncover the molecular aberrations in PC has revealed gene mutations in important oncogenic proteins such as K-Ras, which functions downstream of EGFR. Mutations in K-Ras are involved in >90% of PC cases [4]. Point mutations on this oncogene diminish or abolish its GTPase activity leaving it in the constitutively active state [5]. K-Ras is metabolized via the polyisoprenylation pathway (PP) and efforts to inhibit its maturation and localization have shown anticancer benefit in PC [6]. The PP encompasses a final reversible step that may possibly regulate the proteins' function. Polyisoprenylated protein methyl transferase (PPMTase) esterifies the carboxyl terminus whereas polyisoprenylated methylated protein methyl esterase (PMPMEase) hydrolyzes the ester bond. Substrate analysis of PMPMEase [7,8] led to the synthesis of high affinity, irreversible sulfonyl fluoride inhibitors (Fig. 1) [9]. Meanwhile, polyunsaturated fatty acids (PUFAs), which are known to exhibit anticancer activity, have been shown to inhibit PMPMEase [10]. The decrease in cancer cell viability due to inhibition of PMPMEase with PUFAs, synthetic, and other chemopreventive agents highlights its potential as an anticancer target.

The study aims were three-fold. The first aim was to determine the expression of PMPMEase in PDA and in normal pancreatic tissue in view of the potential to utilize PMPMEase as a possible biomarker and therapeutic target. Second, to design and synthesize polyisoprenylated cysteinyl amide inhibitors (PCAIs), a first-in-class group of compounds to either inhibit PMPMEase and/or disrupt the polyisoprenylation-dependent interactions of the proteins it metabolizes. The design strategy incorporated three key elements to maximize selectivity, biochemical stability and bioavailability. These included, (1) the farnesyl group for high affinity/selective

Abbreviations: EGFR, epidermal growth factor receptor; FTS, S-*t*, *t* farnesylthiosalicylic acid (Salirasib); FTSA, S-*t*, *t* farnesylthiosalicylamide; NT, normal tissue; NAT, normal adjacent tissue; PC, pancreatic cancer; PCAIs, polyisoprenylated cysteinyl amide inhibitors; PDA, pancreatic ductal adenocarcinoma; PMPMEase, polyisoprenylated methylated protein methyl esterase; PMSF, phenylmethylsulfonyl fluoride; PP, polyisoprenylation pathway; PPMTase, polyisoprenylated protein methyl transferase; PUFAs, polyunsaturated fatty acids; RD-PNB, L-N-(4-nitrobenzoyl)-S-*t*, *t*-farnesyl-cysteine methyl ester; TMA, tissue microarray.

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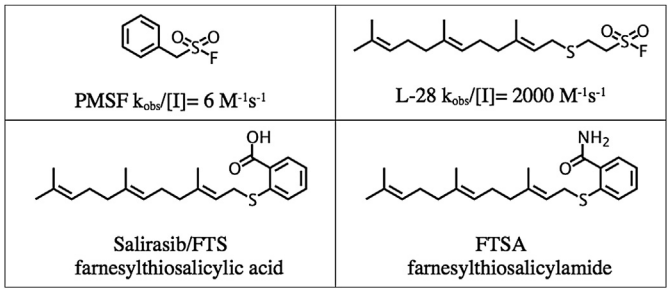


Fig. 1. Structure of PMPMEase (PMSF, L-28) and Ras (FTS and FTSA) inhibitors.

interactions, (2) a substituted amide bioisostere of the scissile ester bond found in the endogenous substrates, and (3) an ionizable appendage group designed to mitigate the excessive hydrophobicity of the farnesyl cysteinyl amide that constitutes the pharmacophore. Finally, to examine the effect of PMPMEase inhibition by PCAIs in PC cell lines.

The potential beneficial impact of these compounds was further investigated by examining the expression of PMPMEase in PDA relative to normal pancreatic tissues that might substantiate the use of the PCAIs as effective targeted therapies. PMPMEase overexpression in PDA would not only contribute to its validation as a putative drug target but also as a biomarker for potential early/companion diagnosis and prognosis. The results strongly suggest possible beneficial outcomes for the continuous development of the PCAIs as a novel class of targeted therapies for PC.

2. Results and discussion

2.1. Chemistry

The design and synthesis of polyisoprenylated sulfonyl fluorides was the first successful attempt at developing inhibitors of PMPMEase [9]. Incorporation of a polyisoprenyl tail increased the inhibitory potency determined using pseudo-first order kinetics by 330-fold compared to the lead compound PMSF. Although these compounds are potent inhibitors, lack of polar groups means that they are very hydrophobic (ClogP ranged from 2.8 to 4.3). Additionally, the highly reactive sulfonyl fluoride functional group is moisture-sensitive. PCAIs address these former limitations by adding an ionizable functional group (pyrrolidine or N-methylpiperazine) to diminish the excessive hydrophobicity. The calculated LogD values ranged from 3.15 to 5.88 at the physiological pH of 7.4 whereas the calculated LogD value for the C15 polyisoprenylated sulfonyl fluoride was 5.04. In addition, the unstable, highly reactive sulfonyl fluoride group that irreversibly inhibits PMPMEase was substituted with an amide group that functions as a more stable bioisostere of the substrate ester bond. The resulting compounds, unlike the sulfonyl fluorides, are reversible PMPMEase inhibitors.

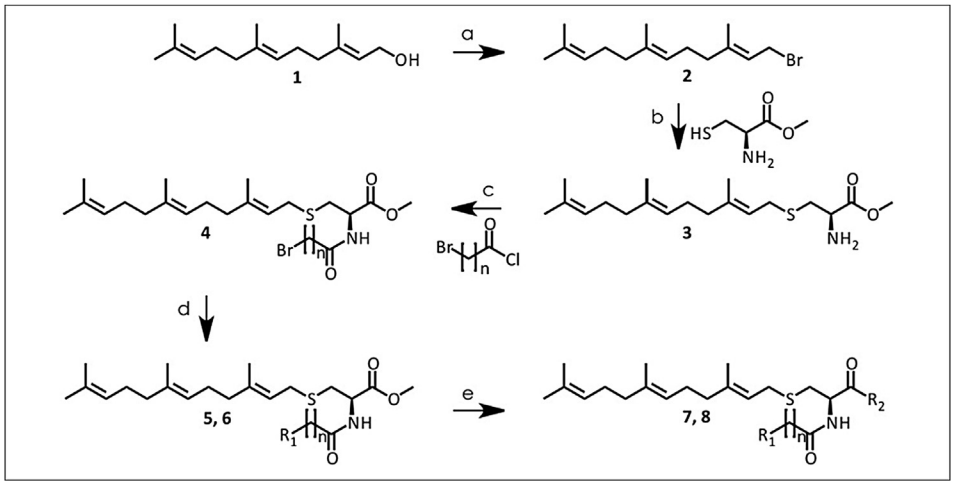
The PCAIs were prepared according to Scheme 1. Briefly, the starting alcohol is first converted to the bromide (**2**) using phosphorous tribromide. The resulting bromide (**2**) was used to alkylate L-cysteine methyl ester to form the alpha amino ester which underwent alkylation with bromoalkynoyl chloride to produce compound **4**. Compound **4** then served as the alkylating agent for the selected amines (pyrrolidine and 1-methylpiperazine) designed to impart solubility, prior to the amidation of the ester function overnight as a neat reaction at 90 °C. However, several low boiling amines and di-substituted amines showed no reaction and required the use of the corresponding acyl chlorides. The acyl chlorides were obtained by hydrolyzing the ester bonds and converting the products to the required acyl chlorides before reaction with the low boiling point amines. Reactions with the amines resulted in compounds **7a**–**g** (pyrrolidines) and **8a**–**g** (1-methylpiperazines).

2.2. Docking analysis

The *in silico* analysis of the PCAIs revealed compounds with AScore docking energies ranging from −17.21 to −13.35 kcal/mol (Table 1). A representative of the PCAIs, **8a** is shown in the active site of PMPMEase in Fig. 2. The pyrrolidine derivatives showed lower docking energies (−17.21 to −14.03 kcal/mol) compared to the N-methylpiperazine derivatives (−15.08 to −13.35 kcal/mol). Compound **7d** had the lowest AScore docking energy of −17.21 kcal/mol versus −14.26 kcal/mol for its N-methylpiperazinyl derivative (**8d**).

2.3. Biological evaluation

The clinicopathologic data of the PC cases are summarized in Table 2. The age range of the donors was 1 month to 78 years with a mean age of 53 years. Intense PMPMEase immunoreactivity was observed in pancreatic tumors. Fig. 3 is a PC TMA showing varying



Scheme 1. Synthesis of polyisoprenylated cysteinyl amide inhibitors (PCAIs) of PMPMease. Reagents: (a) −10 °C, PBr_3, Et_2O (b) TEA, L-cysteine methyl ester, Anh. MeOH (c) 6-bromohexanoyl chloride, TEA, DCM, (d) K_2CO_3, amine, toluene, (e) amine, 90 °C, ON.