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Inhibitors of insulin-like growth factor-1 receptor tyrosine kinase are preferentially cytotoxic to nutrient-deprived pancreatic cancer cells

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ABSTRACT

Chronic deprivation of nutrients is rare in normal tissues, however large areas of tumor are nutrient-starved and hypoxic due to a disorganized vascular system. Some cancers show an inherent ability to tolerate severe growth conditions. Therefore, we screened chemical compounds to identify cytotoxic agents that function preferentially in nutrient-deprived conditions. We found that AG1024, a specific inhibitor of insulin-like growth factor-1 receptor tyrosine kinase (IGF-1R), showed preferential cytotoxicity to human pancreatic cancer cells in nutrient-deprived conditions relative to cells in nutrient-sufficient conditions. The cytotoxicity of I-OMe-AG538 (another specific inhibitor of IGF-1R kinase) was also enhanced in nutrient-deprived cells. In addition, AG1024 and I-OMe-AG538 potently inhibited IGF-1R activation to nutrient-deprived cells. In contrast, conventional chemotherapeutic drugs, as well as inhibitors of PDGFR and EGFR kinases, elicited weak cytotoxicity. These data indicate that nutrient-deprived human pancreatic cancer cells have increased sensitivity to inhibition of IGF-1R activation. IGF-1R inhibitors offer a promising strategy for anticancer therapeutic approaches that are oriented toward tumor microenvironment.

Patients diagnosed with pancreatic cancer, an aggressive disease with the lowest 5-year survival rates of all cancers, develop metastases rapidly and die within a short period of time after diagnosis [1,2]. Pancreatic cancer is largely resistant to almost all known chemotherapeutic agents, including 5-fluorouracil, paclitaxel and doxorubicin; surgery is the only current treatment modality that offers any prospect of potential cure. Clearly, there is a dire need for new therapeutic alternatives that improve clinical outcome for pancreatic cancer patients.

Tumor microenvironment exerts an important influence on cancer physiology. The disorganized vascular system in a tumor often results in large areas of tumor starved for nutrients and oxygen. Pancreatic cancers in particular, which are characterized as hypovascular tumors, show an inherent ability to tolerate severe growth conditions. Certain human pancreatic cancer cell lines, including PANC-1, AsPC-1, BxPC-3 and KP-3, exhibit marked environmental tolerance and can survive for prolonged periods of time in nutrient-deprived conditions. Because tolerance of these cancer cells to nutrient starvation has been associated with the activity of protein kinase B (PKB)/Akt [3], it has been hypothesized that agents that diminish such tolerance could function as anticancer agents [4–7].

Insulin-like growth factors-1 (IGF-1) and -2 (IGF-2) are involved in the pathophysiology of a wide range of human neoplasias due to the mitogenic and antiapoptotic properties mediated by their type 1 receptor (IGF-1R) [8]. IGF-1R is a tetrameric transmembrane receptor tyrosine kinase composed of two α and β subunits. The extracellular α subunit is responsible for ligand binding, whereas the β subunit consists of a transmembrane domain and an intracellular tyrosine kinase domain [9,10]. Ligand binding activates the intrinsic receptor tyrosine kinase, resulting in trans-β subunit autophosphorylation and stimulation of PI3K-AKT-TOR and RAF-MAPK signaling pathways. In addition to cell proliferation, activation of IGF-1R has been reported to stimulate cell survival, transformation, metastasis and angiogenesis [11]. Targeted inhibition of IGF-1R signaling has been shown to result in impressive anti-neoplastic activity in many *in vitro* and *in vivo* models of common human cancers. IGF-1R small interfering RNAs [12], anti-receptor antibodies [13,14], a IGF-1-like competitive peptide antagonist [15], a dominant-negative IGF-1R [16–18] and small-molecule IGF-1R tyrosine kinase inhibitors [19,20] have all been found to interfere with cell growth and proliferation. IGF-1R is therefore regarded as an attractive potential target in the development of new drugs to treat malignant tumors.

In this study, we screened chemical compounds to identify agents that preferentially reduce the survival of nutrient-deprived human pancreatic cancer PANC-1 cells. The screen identified IGF-1R inhibitors, which function as cytotoxic agents preferentially on human pancreatic cancer cells in nutrient-deprived conditions.

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Materials and methods

Materials. Antibodies used in Western blotting included anti-IGF-1Rβ (sc-713), anti-Erk 1 (sc-93) and anti-phospho-Erk (sc-7383) from Santa Cruz Biotechnology (Santa Cruz, CA); anti-α-tubulin (T5168) from Sigma–Aldrich (St. Louis, MO); and anti-Akt (#9272), anti-phospho-Akt (Ser 473) (#9271), anti-phospho-Akt (Thr 308) (#9275) and anti-phospho-IGF-1R (#3021) from Cell Signaling Technology (Denvers, MA). Recombinant human IGF-1 was from R&D Systems (Minneapolis, MN). AG1024, AG1296, AG1478 and I-OMe-AG538 were obtained from Calbiochem (Madison, WI). Doxorubicin hydrochloride, fluorouracil, paclitaxel and mitomycin C were from Sigma. The SCADS inhibitor kit I consisting of 79 chemical inhibitors with ~60 different targets was kindly provided by the Screening Committee on Anticancer Drugs (Japan).

Cells and culture. Human pancreatic cancer cell lines PANC-1, Capan-1, MIA Paca-2, BxPC-3 and PSN-1 were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown at 37 °C with 5% CO_2 in Dulbecco's modified Eagle medium (DMEM; Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Tissue Culture Biologicals, Tulare, CA), 100,000 U/L penicillin G, and 100 mg/L streptomycin. Nutrient starvation was achieved by culturing the cells in nutrient-deprived medium (NDM) as previously described [3–7]. Briefly, the composition of the NDM was as follows: 265 mg/L $CaCl_2{\cdot}H_2O$, 400 mg/L KCl, 200 mg/L $MgSO_4{\cdot}7H_2O$, 6400 mg/L NaCl, 163 mg/L $NaH_2PO_4{\cdot}2H_2O$, 0.1 mg/L $Fe(NO_3)_3{\cdot}9H_2O$, 5 mg/L phenol red, 100,000 U/L penicillin G, 100 mg/L streptomycin, 25 mmol/L HEPES buffer (pH 7.4), and MEM vitamin solution (Invitorogen, Carlsbad, CA); the final pH was adjusted to 7.4 with 10% $NaHCO_3$.

Preferential cytotoxicity in nutrient-deprived conditions. PANC-1 cells (2.5×10^4 cells/well) in 96-well plates were cultured in DMEM for 24 h. The cells were washed with PBS and the medium was replaced with either fresh DMEM or NDM. Test samples were added to the well and cells were cultured for 24 h. Cytotoxicity was determined using the MTT assay [21].

Preparation of cell lysate and Western blotting. PANC-1 cells (5×10^5) in 35-mm dishes were incubated in DMEM for 24 h. The cells were washed with PBS and the medium was replaced with either fresh DMEM or NDM. AG1024 or I-OMe-AG538 was added to each dish and the cells were incubated for 1 h prior to stimulation with 50 ng/ml IGF-1 for 10 min. The cells were washed twice with ice-cold PBS containing 100 μm Na_3VO_4 and then lysed in a lysis buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerolphosphate, 1 mM Na_3VO_4, pH 7.5, and 25 μg/ml each of antipain, leupeptin, and pepstatin). Equal amounts of protein extract were separated by SDS–polyacrylamide gel electrophoresis, transferred onto Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA) and probed with anti-IGF-1R, anti-phospho-IGF-1R, anti-Akt, anti-phospho-Akt (Thr 308), anti-phospho-Akt (Ser 473), anti-Erk 1,

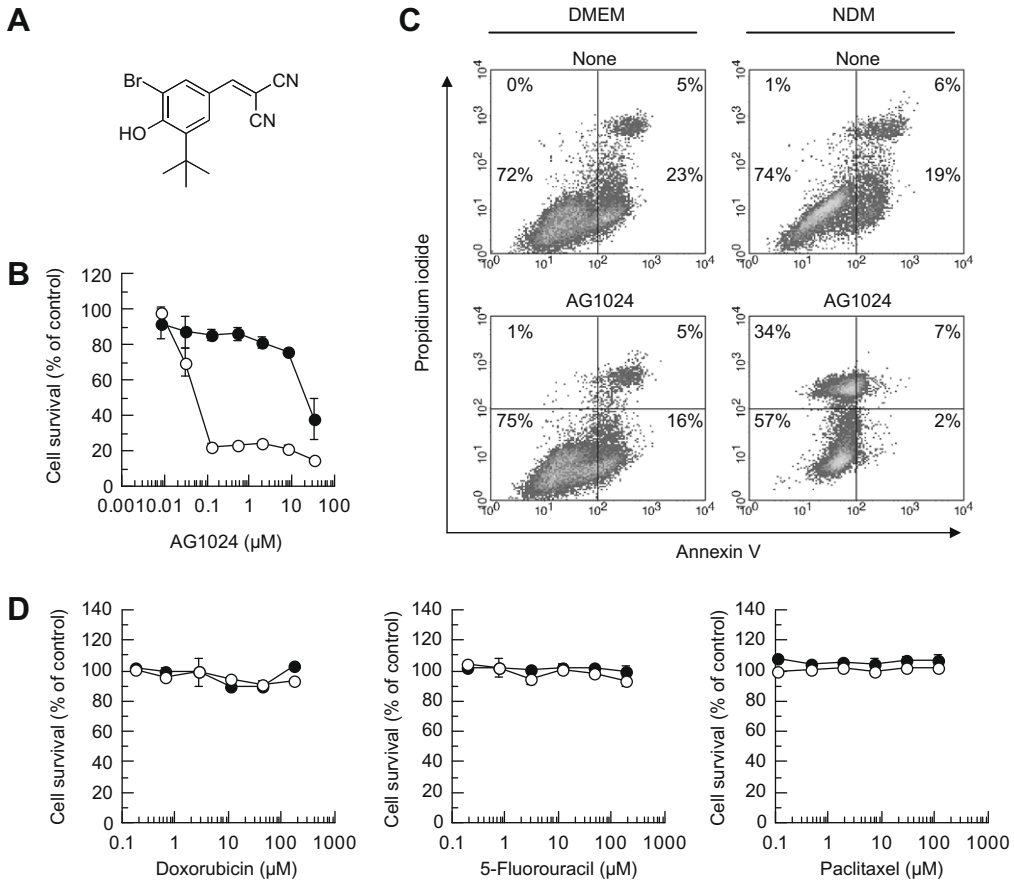

Fig. 1. Effect of AG1024 on survival of PANC-1 cells in nutrient-deprived conditions. (A) Structure of AG1024. (B) Effect of AG1024 on PANC-1 cell viability in normal medium, DMEM (●) and nutrient-deprived medium, NDM (○). PANC-1 cells incubated in DMEM for 24 h. The cells were then washed with PBS and the medium was replaced with either fresh DMEM or NDM. The indicated concentrations of AG1024 were added to each well and the cells were incubated for 24 h. Cell viability was determined using the MTT assay. (C) Flow cytometric analysis of AG1024-treated PANC-1 cells. PANC-1 cells were cultured with 0.3 μM AG1024 in DMEM or NDM for 12 h. The cells were stained with annexin V-FITC and propidium iodide according to instructions for the apoptosis detection kit and then analyzed using a flow cytometer. (D) Effect of conventional anticancer drugs on survival of PANC-1 cells in nutrient-deprived conditions. PANC-1 cells were incubated with indicated concentrations of doxorubicin, 5-fluorouracil and paclitaxel in DMEM (●) or NDM (○) for 24 h.

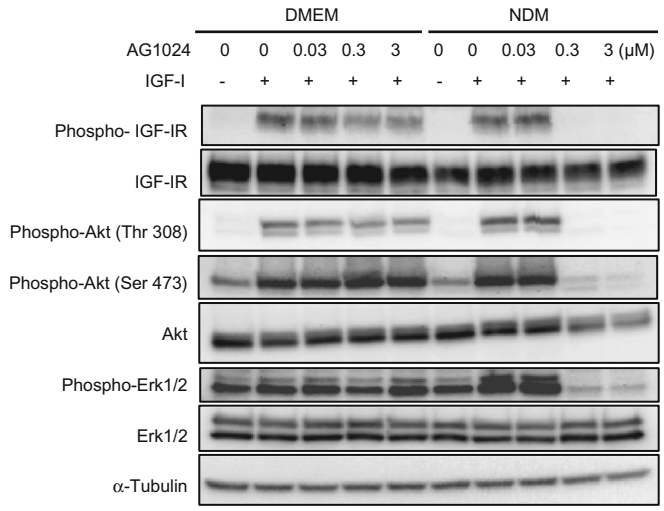

Fig. 2. Effect of AG1024 on IGF-1R activation. PANC-1 cells incubated in DMEM for 24 h were washed with PBS and the medium was replaced with either fresh DMEM or NDM. The cells were incubated with the indicated concentration of AG1024 for 1 h before stimulation with 50 ng/ml IGF-1 for 10 min. Cell lysates were resolved using SDS–PAGE and transferred to membranes for western blotting with specific antibodies.

anti-phospho-Erk, or anti-tubulin antibodies. Horseradish peroxidase-linked anti-rabbit IgG or anti-mouse IgG antibodies were used as secondary antibodies (GE Healthcare, Piscataway, NJ). The blots were developed using ECL reagent according to the manufacturer's instructions (GE healthcare).

Flow cytometric analysis. PANC-1 cells (5×10^5) in 60-mm dishes were incubated in DMEM for 24 h. The cells were washed with PBS and the medium was replaced with either fresh DMEM or NDM. AG1024 (0.3 μM) was then added and the cells were cultured for 12 h. The cells were incubated with annexin V-FITC and propidium iodide using an annexin V-FITC apoptosis detection kit (Biovision Research Products, Mountain View, CA) and analyzed using a flow cytometer (FACSCalibur; BD Biosciences, Franklin Lakes, NJ).

Statistical analysis. All data are representative of three independent experiments with similar results. The statistical data are expressed as mean ± SD using descriptive statistics.

Results

AG1024 is preferentially cytotoxic to human pancreatic cancer PANC-1 cells in nutrient-deprived conditions

To identify cytotoxic agents that function preferentially on nutrient-deprived cells, we tested the cytotoxic effects of small-molecule inhibitors in the SCADS inhibitors kit I. As shown in Table S1, a specific inhibitor of IGF-1R tyrosine kinase, termed AG1024, was found to be cytotoxic to PANC-1 cells in nutrient-deprived medium (NDM), but not in normal medium (DMEM). The structure of AG1024 [22], otherwise known as 2-(3-bromo-5-*t*-butyl-4-hydroxybenzylidene)malonitrile, is shown in Fig. 1A. To determine the dose–response relationship of AG1024 cytotoxicity, PANC-1

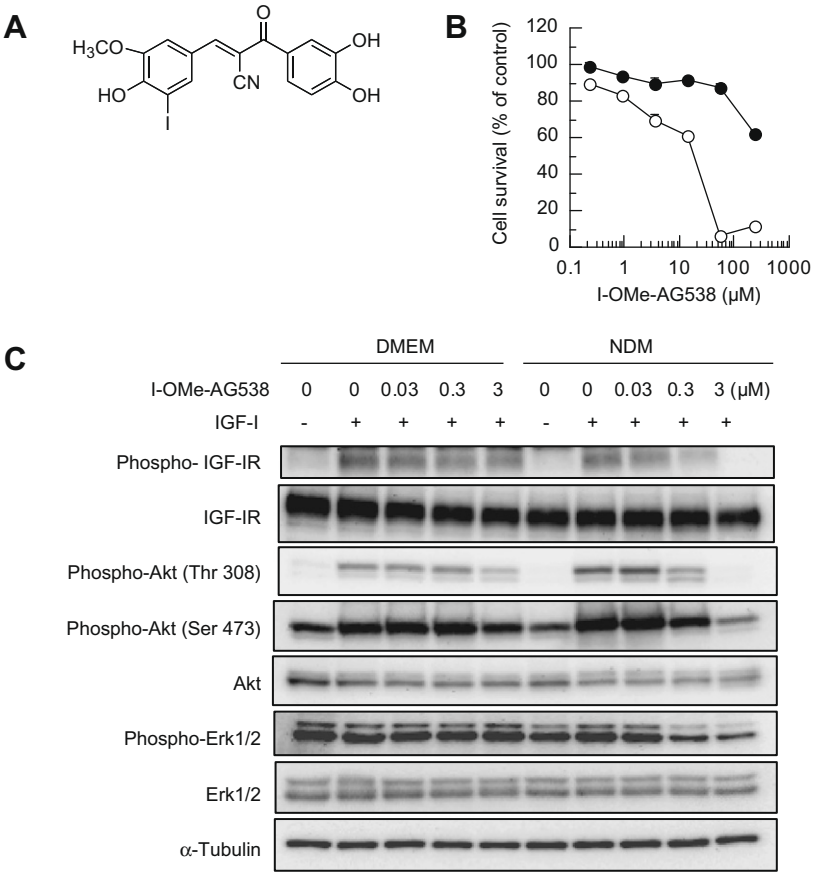

Fig. 3. Effect of I-OMe-AG538 on survival of nutrient-deprived PANC-1 cells. (A) Structure of I-OMe-AG538. (B) Effect of I-OMe-AG538 on PANC-1 cell viability in DMEM (●) or NDM (○). PANC-1 cells were incubated with the indicated concentrations of I-OMe-AG538 in DMEM or NDM for 24 h. (C) Effect of I-OMe-AG538 on IGF-1R activation. PANC-1 cells in DMEM or NDM were incubated with the indicated concentration of I-OMe-AG538 for 1 h before stimulation with 50 ng/ml IGF-1 for 10 min. Cell lysates were resolved using SDS–PAGE and transferred to membranes for western blotting with specific antibodies.

cells grown in NDM or DMEM were exposed to increasing concentrations of AG1024 for 24 h (Fig. 1B). The cytotoxic effect of AG1024 was more than 100 times greater on nutrient-deprived PANC-1 cells (NDM IC_{50} 0.055 μM) relative to cells in nutrient-sufficient medium (DMEM IC_{50} 21 μM). In DMEM, 0.3 μM AG1024 did not induce any significant PANC-1 cell death as determined using propidium iodide and annexin V staining and flow cytometry (Fig. 1C). In contrast, 34% of the cells grown in NDM and treated with the same concentration of AG1024 showed propidium iodide-positive/annexin V-negative staining. We compared the cytotoxicity of AG1024 to that of several conventional anticancer drugs, including doxorubicin, 5-fluorouracil and paclitaxel, in PANC-1 cells grown in NDM versus DMEM (Fig. 1D). The cytotoxicity of doxorubicin, 5-fluorouracil and paclitaxel on PANC-1 cells grown in either medium for 24 h was significantly weaker than AG1024. These results demonstrate clearly that AG1024 exhibits preferential cytotoxicity to nutrient-deprived PANC-1 cells.

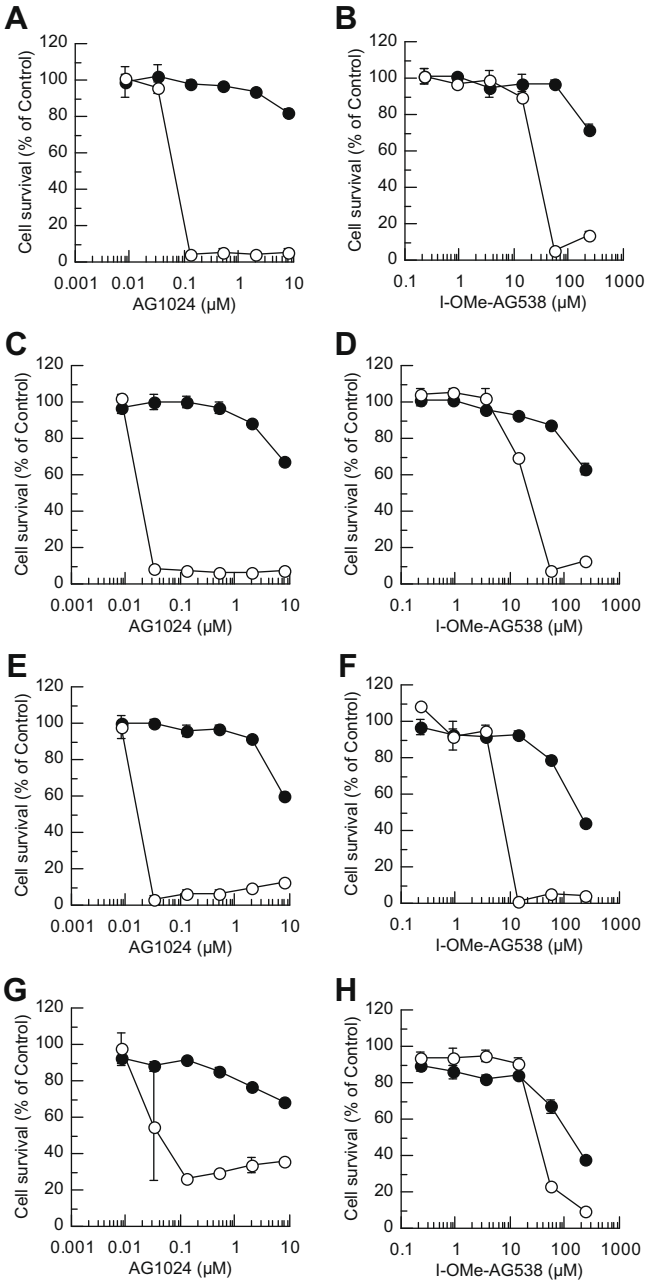

Fig. 4. Effect of AG1024 and I-OMe-AG538 on various human pancreatic cancer cell lines in nutrient-deprived conditions. Human pancreatic cancer cells were incubated with indicated concentrations of AG1024 or I-OMe-AG538 in DMEM (●) or NDM (○) for 24 h. A,B, Capan-1; C,D, MIA Paca-2; E,F, BxPC-3; G,H, PSN-1.

AG1024 inhibits activation of IGF-1R in nutrient-deprived PANC-1 cells

Because AG1024 is a specific inhibitor of IGF-1R kinase, we examined the effect of AG1024 on IGF-1-mediated phosphorylation of IGF-1R in PANC-1 cells grown in different media (Fig. 2). While addition of 0.3 μM AG1024 to PANC-1 cells grown in NDM resulted in a complete inhibition of IGF-1R autophosphorylation, phosphorylation of IGF-1R was only weakly inhibited in cells grown in DMEM with 10-fold higher concentrations of AG1024 (3 μM). AG1024 also inhibited the phosphorylation of Akt (Thr 308), Akt (Ser 473) and Erk, which normally occur as a result of IGF-1R activation. These results demonstrate that AG1024 is a potent inhibitor of IGF-1R activation in nutrient-deprived PANC-1 cells.

I-OMe-AG538 is preferentially cytotoxic to nutrient-deprived PANC-1 cells

In testing whether other IGF-1R inhibitors also functioned preferentially in nutrient-deprived cells, we found that I-OMe-AG538 [23] (another specific inhibitor of IGF-1R, Fig. 3A) also was more cytotoxic to cells in nutrient-deprived medium relative to those in nutrient-sufficient conditions (Fig. 3B). The effect of I-OMe-AG538 on IGF-1R activation in nutrient-deprived cells was similar to AG1024, in that it blocked phosphorylation of IGF-1R, Akt and Erk (Fig. 3C). Our results demonstrate clearly that the IGF-1R inhibitors AG1024 and I-OMe-AG538 both inhibit IGF-1R-mediated signaling and are preferentially cytotoxic to nutrient-deprived PANC-1 cells.

Inhibitors of IGF-1R show preferential cytotoxicity to various human pancreatic cancer cells lines in nutrient-deprived conditions

To determine whether inhibitors of IGF-1R kinase exhibit preferential cytotoxicity to other nutrient-deprived human pancreatic cancer cell lines, we examined the cytotoxic effects of AG1024 and I-OMe-AG538 on Capan-1, MIA Paca-2, BxPC-3, and PSN cells (Fig. 4). AG1024 and I-OMe-AG538 were significantly more cytotoxic to all four human pancreatic cancer cell lines in NDM relative to DMEM, indicating that the cytotoxicity of IGF-1R kinase inhibitors is likely to occur in nutrient-deprived human pancreatic cancer cells. To understand the specificity of IGF-1R kinase inhibitors, we also examined the cytotoxic effects of other representative receptor tyrosine kinase inhibitors (Fig. S1). The cytotoxicities of AG1296 (a PDGFR kinase inhibitor) [24] and AG1478 and PD168393 (EGFR kinase inhibitors) [25–27] were significantly reduced, relative to IGF-1R inhibitors, in both nutrient-deprived and -fed PANC-1 cells. These results indicate that specific inhibition of IGF-1R kinase is important in promoting preferential cytotoxicity in nutrient-starved human pancreatic cancer cells.

Discussion

Tumor microenvironment strongly influences tumor growth and progression. Many aspects of physiology that differentiate solid tumors from normal tissues arise from differences in vasculature. Disorganized vascular systems in tumors result in large areas of tumor exposed to nutrient starvation and hypoxic conditions. In addition, due to the unregulated growth of tumor cells caused by genetic and epigenetic alterations, cells proliferate more rapidly than normal cells and nutrient and oxygen demands often exceeds supply [28–30]. Cancer cells, in particular highly aggres-

sive tumors such as pancreatic cancer that are relatively hypovascular, are able to survive even in conditions of low nutrients and low oxygen supply. Since chronic nutrient deprivation seldom occurs in normal tissues, one strategy for anticancer agent development is to target cancer cells growing in nutrient-deprived conditions. Thus, we screened to identify cytotoxic agents that function preferentially in nutrient-deprived cells.

We found that AG1024, a specific inhibitor of IGF-1R kinase, showed preferential cytotoxicity to human pancreatic cancer PANC-1 cells grown in nutrient-deprived medium. Conventional chemotherapeutic drugs such as doxorubicin, 5-fluorouracil and paclitaxel, were only weakly cytotoxic to nutrient-deprived PANC-1 cells, suggesting that AG1024 may be a unique and attractive starting compound in the development of an antitumor agent. AG1024 has been reported to induce apoptosis in human breast cancer MCF-7 cells [31]. In our present study, flow cytometric analysis showed that AG1024 increased propidium iodide staining without annexin V of nutrient-deprived PANC-1 cells. Kigamicin D and (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan induced necrosis in nutrient-deprived cells [4,32]. Therefore, AG1024 may induce necrosis under nutrient starvation. I-OMe-AG538, another IGF-1R kinase inhibitor that differs in structure from AG1024, was also cytotoxic to nutrient-deprived PANC-1 cells. These IGF-1R kinase inhibitors also were cytotoxic to other nutrient-deprived human pancreatic cancer cell lines, including Capan-1, MIA Paca-2, BxPC-3 and PSN.

IGF-1 binding to the IGF-1R results in activation of receptor tyrosine kinases that stimulates signaling through intracellular networks, including PI3K-AKT-TOR and RAF-MAPK, which then promote cell proliferation and inhibit apoptosis. We found that the IGF-1R kinase inhibitors AG1024 and I-OMe-AG538 blocked phosphorylation of IGF-1R by IGF-1 preferentially in cells cultured in nutrient-deprived conditions relative to those in nutrient-sufficient conditions. These IGF-1R kinase inhibitors also suppressed phosphorylation of Akt and Erk, demonstrating that activation of pathways downstream of the IGF-1R were also blocked in nutrient-deprived conditions.

Unlike AG1296 (a PDGFR kinase inhibitor) or AG1478 and PD168393 (EGFR kinase inhibitors), which are less cytotoxic in nutrient-deprived PANC-1 cells, preferential inhibition of IGF-1 signaling by IGF-1R kinase inhibitors suggests that this pathway may play an important role in cell survival in stress conditions such as nutrient deprivation. The Akt pathway, which functions downstream of IGF-1R, plays a critical role in the proliferation, survival, motility, morphology and therapeutic resistance of cancer cells [33,34]. Because Akt has been demonstrated to regulate cell survival in various stress conditions, including nutrient deprivation, this kinase is viewed as a promising target for cancer therapeutics. Akt inhibitors have been developed including PX-316, which shows antitumor activity against human MCF-7 breast cancer and HT-29 colon cancer xenografts in mice [35]. Thus, part of the preferential cytotoxicity of IGF-1R kinase inhibitors in nutrient-deprived conditions may be due to inhibition of Akt activation.

The IGF-1 receptor is universally expressed in various hematologic and solid tumor cells. NVP-ADW742, another specific inhibitor of IGF-1R kinase, has been shown to be a significant antitumor agent in an orthotopic xenograft multiple myeloma model [20]. Oral administration of the IGF-1R kinase-specific inhibitor NVP-AEW541 has been shown to inhibit IGF-1R signaling in tumors and to reduce tumor growth in a xenograft fibrosarcoma model [19]. The potent cytotoxicity of AG1024 and I-OMe-AG538 to pancreatic cancer cell lines deprived of nutrients (simulating a tumor microenvironment) makes IGF-1R a promising target for new drugs that may be developed to treat a broad spectrum of malignant tumors.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.01.065.

References

[1] D. Li, K. Xie, R. Wolff, J.L. Abbruzzese, Pancreatic cancer, Lancet 363 (2004) 1049–1057.
[2] S. Shore, D. Vimalachandran, M.G. Raraty, P. Ghaneh, Cancer in the elderly: pancreatic cancer, Surg. Oncol. 13 (2004) 201–210.
[3] K. Izuishi, K. Kato, T. Ogura, T. Kinoshita, H. Esumi, Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy, Cancer Res. 60 (2000) 6201–6207.
[4] J. Lu, S. Kunimoto, Y. Yamazaki, M. Kaminishi, H. Esumi, D. Kigamicin, A novel anticancer agent based on a new anti-austerity strategy targeting cancer cells' tolerance to nutrient starvation, Cancer Sci. 95 (2004) 547–552.
[5] S. Kunimoto, J. Lu, H. Esumi, Y. Yamazaki, N. Kinoshita, Y. Honma, M. Hamada, M. Ohsono, M. Ishizuka, T. Takeuchi, D. Kigamicins, Novel antitumor antibiotics. I. Taxonomy, isolation, physico-chemical properties and biological activities, J. Antibiot. 56 (2003) 1004–1011.
[6] S. Kunimoto, T. Someno, Y. Yamazaki, J. Lu, H. Esumi, H. Naganawa, D. Kigamicins, Novel antitumor antibiotics. II. Structure determination, J. Antibiot. 56 (2003) 1007–1012.
[7] H. Esumi, J. Lu, Y. Kurashima, T. Hanaoka, Antitumor activity of pyrvinium pamoate, 6-(dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl-1H-pyrrol-3-yl)ethenyl]-1-methyl-quinolinium pamoate salt, showing preferential cytotoxicity during glucose starvation, Cancer Sci. 95 (2004) 685–690.
[8] M.N. Pollak, E.S. Schernhammer, S.E. Hankinson, Insulin-like growth factors and neoplasia, Nat. Rev. Cancer 4 (2004) 505–518.
[9] A. Ullricha, J. Schlessingerb, Signal transduction by receptors with tyrosine kinase activity, Cell 61 (1990) 203–212.
[10] P.V.D. Geer, T. Hunter, R.A. Lindberg, Receptor protein-tyrosine kinases and their signal transduction pathways, Annu. Rev. Cell Biol. 10 (1994) 251–337.
[11] R. Baserga, The contradictions of the insulin-like growth factor 1 receptor, Oncogene 19 (2000) 5574–5581.
[12] E.A. Bohula, A.J. Salisbury, M. Sohail, M.P. Playford, J. Riedemann, E.M. Southern, V.M. Macaulay, The efficacy of small interfering RNAs targeted to the type 1 insulin-like growth factor receptor (IGF1R) is influenced by secondary structure in the IGF1R transcript, J. Biol. Chem. 278 (2003) 15991–15997.
[13] E.K. Maloney, J.L. McLaughlin, N.E. Dagdigian, L.M. Garrett, K.M. Connors, X.-M. Zhou, W.A. Blättler, T. Chittenden, R. Singh, An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation, Cancer Res. 63 (2003) 5073–5083.
[14] D. Burtrum, Z. Zhu, D. Lu, D.M. Anderson, M. Prewett, D.S. Pereira, R. Bassi, R. Abdullah, A.T. Hooper, H. Koo, X. Jimenez, D. Johnson, R. Apblett, P. Kussie, P. Bohlen, L. Witte, D.J. Hicklin, D.L. Ludwig, A fully human monoclonal antibody to the insulin-like growth factor I receptor blocks ligand-dependent signaling and inhibits human tumor growth in vivo, Cancer Res. 63 (2003) 8912–8921.
[15] P. Hayry, M. Myllarniemi, E. Aavik, S. Alatalo, P. Aho, S. Yilmaz, A.R. Sokolowski, G. Cozzone, B. Jameson, R. Baserga, Stabile D-peptide analog of insulin-like growth factor-1 inhibits smooth muscle cell proliferation after carotid ballooning injury in the rat, FASEB J. 9 (1995) 1336–1344.
[16] D. Sachdev, J.S. Hartell, A.V. Lee, X. Zhang, D. Yee, A dominant negative type I insulin-like growth factor receptor inhibits metastasis of human cancer cells, J. Biol. Chem. 279 (2004) 5017–5024.
[17] C.-T. Lee, K.-H. Park, Y. Adachi, J.Y. Seol, C.-G. Yoo, Y.W. Kim, S.K. Han, Y.-S. Shim, K. Coffee, M.M. Dikov, D.P. Carbone, Recombinant adenoviruses expressing dominant negative insulin-like growth factor-I receptor demonstrate antitumor effects on lung cancer, Cancer Gene. Ther. 10 (2003) 57–63.
[18] Y. Min, Y. Adachi, H. Yamamoto, H. Ito, F. Itoh, C.-T. Lee, S. Nadaf, D.P. Carbone, K. Imai, Genetic blockade of the insulin-like growth factor-I receptor: a promising strategy for human pancreatic cancer, Cancer Res. 63 (2003) 6432–6441.
[19] C. García-Echeverría, M.A. Pearson, A. Marti, T. Meyer, J. Mestan, J. Zimmermann, J. Gao, J. Brueggen, H.-G. Capraro, R. Cozens, D.B. Evans, D.

Fabbro, P. Furet, D.G. Porta, J. Liebetanz, G. Martiny-Baron, S. Ruetz, F. Hofmann, In vivo antitumor activity of NVP-AEW541—a novel, potent, and selective inhibitor of the IGF-IR kinase, Cancer Cell 5 (2004) 231–239.
[20] C.S. Mitsiades, N.S. Mitsiades, C.J. McMullan, V. Poulaki, R. Shringarpure, M. Akiyama, T. Hideshima, D. Chauhan, M. Joseph, T.A. Libermann, C. García-Echeverría, M.A. Pearson, F. Hofmann, K.C. Anderson, Andrew L. Kung, Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors, Cancer Cell 5 (2004) 221–230.
[21] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[22] B. Wen, E. Deutsch, E. Marngoni, V. Frascona, L. Maggiorella, B. Abdulkarim, N. Chavaudra, J. Bourhis, Tyrphostin AG 1024 modulates rediosensitivity in human breast cancer cells, Br. J. Cancer 85 (2001) 2017–2021.
[23] G. Blum, A. Gazit, A. Levitzki, Substrate competitive inhibitors of IGF-1 receptor kinase, Biochemistry 39 (2000) 15705–15712.
[24] L.M. Strawn, G. McMahon, H. App, R. Schreck, W.R. Kuchler, M.P. Longhi, T.H. Hui, C. Tang, A. Levitzki, A. Gazit, I. Chen, G. Keri, L. Orfi, W. Risau, I. Flamme, A. Ullrich, K.P. Hirth, L.K. Shawver, Flk-1 as a target for tumor growth inhibition, Cancer Res. 56 (1996) 3540–3545.
[25] A. Levitzki, A. Gazit, Tyrosine kinase inhibition: an approach to drug development, Science 267 (1995) 1782–1788.
[26] R. Bose, H. Molina, A.S. Patterson, J.K. Bitok, B. Periaswamy, J.S. Bader, A. Pandey, P.A. Cole, Phosphoproteomic analysis of Her2/neu signaling and inhibition, Proc. Natl. Acad. Sci. USA 103 (2006) 9773–9778.
[27] D.W. Fry, A.J. Bridges, W.A. Denny, A. Doherty, K.D. Greis, J.L. Hicks, K.E. Hook, P.R. Keller, W.R. Leopold, J.A. Loo, D.J. McNamara, J.M. Nelson, V. Sherwood, J.B. Smaill, S. Trumpp-Kallmeyer, E.M. Dobrusin, Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor, Proc. Natl. Acad. Sci. USA 95 (1998) 12022–12027.
[28] C.V. Dang, G.L. Semenza, Oncogenic alterations of metabolism, Trends Biochem. Sci. 24 (1999) 68–72.
[29] R.M. Southerland, Cell and environment interactions in tumor microregions: the multicell spheroid model, Science 240 (1988) 178–184.
[30] G. Helmlinger, F. Yuan, M. Dellian, R.K. Jain, Interstitial pH and pO_2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation, Nat. Med. 3 (1997) 177–182.
[31] P. Marcelina, G. Aviv, L. Alexander, W. Efrat, L. Derek, Specific inhibitor of insulin-like growth factor-1 and insulin receptor tyrosine kinase activity and biological function by tyrphostins, Endocrinology 138 (1997) 1427–1433.
[32] S. Awale, F. Li, H. Onozuka, H. Esumi, Y. Tezuka, S. Kadota, Constituents of Brazilian red propolis and their preferential cytotoxic activity against human pancreatic PANC-1 cancer cell line in nutrient-deprived condition, Bioorg. Med. Chem. 16 (2008) 181–189.
[33] A. Toker, M. Yoeli-Lerner, Akt signaling and cancer: surviving but not moving on, Cancer Res. 66 (2006) 3963–3966.
[34] A.G. Bader, S. Kang, L. Zhao, P.K. Vogt, Oncogenic PI3K deregulates transcription and translation, Nat. Rev. Cancer 5 (2005) 921–929.
[35] E.J. Meuillet, N. Ihle, A.F. Baker, J.M. Gard, C. Stamper, R. Williams, A. Coon, D. Mahadevan, B.L. George, L. Kirkpatrick, G. Powis, In vivo molecular pharmacology and antitumor activity of the targeted Akt inhibitor PX-316, Oncol. Res. 14 (2004) 513–527.