1. The incidence and distribution of K-ras mutations in pancreas cancer differs by country. The relative frequency of mutant K-ras (G12 and R13) differs between countries. Mutations at codon 12 were found only in patients from the US and India. Mutations at codon 16 are more frequent than previously reported in pancreas cancer (10% vs 5%).

2. A new Ras E76 mutation was identified and found to be more prevalent in late-stage cancers. The E76 mutation was frequently detected along with a hot spot G12 mutation in < 10% of samples with mutant K-ras.

3. Ras E76 mutations synergize with Ras G12 mutations to exacerbate the aggressive tumor growth phenotype in preclinical models. Based on the preclinical results shown above, these double mutations may act synergistically to drive cancer progression.

Fig. 4. Residues 61, 62 and 76 affect domains of Ras function.

Fig. 5. Ras mutations at residues G12 and E76 have synergistic effects on GTP binding. The E76 mutation synergizes with the G12 mutation to restore the GTPase activity, which is lost in the G12E mutant.

Fig. 6. Tumorigenicity assay with BALB/c-3T3 nude mice

Table 1. Ras mutations cause transforming phenotypes in vitro

The E76 mutation was frequently detected along with a hot spot G12 mutation in < 10% of samples with mutant K-ras. Based on the preclinical results shown above, these double mutations may act synergistically to drive cancer progression.

Fig. 7. Mutations in ras codon E76 are more frequently observed in late-stage disease.

Conclusions
1. The incidence and distribution of K-ras mutations in pancreatic cancer differ in patients from different countries, underscoring the essential role of direct genotyping to personalize cancer treatment paradigms.
2. A new Ras E76 mutation was identified and found to be more prevalent in late-stage cancers.
3. Ras E76 mutations synergize with the G12 mutation to restore the GTPase activity, which is lost in the G12E mutant.
4. Ras E76 mutations represent a new cancer biomarker and target for cancer immunotherapy.
DNA encoding target protein antigens is engineered into a yeast expression plasmid. The target antigens are markers of diseased cells and can be conserved viral proteins, mutated pathogens are whole, heat-killed recombinant Tarmogens. Cancer immunotherapy with yeast-based Tarmogens is a potent activation of antigen-specific cellular immune responses against cancer cells or virally infected cells. Repeated dosing with Tarmogens further increases the therapeutic benefit from the Tarmogen is driven by the targeted activation of the immune system. Tarmogens activate killer T cells (causing activation of these cells). Tarmogens are also digested in endosomes, and the product-associated peptides are loaded into the peptide-MHC I receptor complex. These small peptides are loaded into newly folded MHC class I receptors in the secretory pathway of the APC. The intracellular content of GTP-bound Ras protein, a marker of Ras activation, was assayed with cells transfected to express extra copies of wild type Ras, or Ras with single or double mutations. The intracellular content of GTP-bound Ras protein was assayed with cells transfected to express extra copies of wild type Ras, or Ras with single or double mutations. For more information, visit www.globeimmune.com.

Cancer immunotherapy with yeast-based Tarmogens

Tarmogens are whole, heat-killed recombinant Saccharomyces cerevisiae yeast modified to express one or more protein targets that stimulate the immune system against diseased cells. The target antigens are markers of diseased cells and can be conserved viral proteins, mutated proteins unique to cancer cells, or proteins over-expressed in cancer. To create a new Tarmogen, DNA encoding target protein antigens is engineered into a yeast expression plasmid. The heat-inactivated yeast, with the target protein inside, is administered as the final Tarmogen product. Tarmogens stimulate the innate and antigen-specific immune systems to produce a highly specific and potent T cell response against the diseased cell, with little or no impact on healthy cells.

Tarmogens are administrated subcutaneously and are avidly taken up by antigen-presenting cells (APC), such as dendritic cells and macrophages in a process mediated by Toll-like receptors (TLRs). Upon binding to the TLRs found on the cell surface, uptake of Tarmogens activates the APCs and results in their migration to lymph nodes and their production of immune-stimulating cytokines. Tarmogens are digested inside APCs, within hours and the target antigens are presented by MHC class I and II receptors for antigen presentation to CD8+ T cells (causing activation of these cells) and CD4+ Helper T cells (causing activation of these cells).

Therapeutic benefits from the Tarmogen is driven by the targeted activation of the immune system. Tarmogens activate killer T cells capable of locating and destroying the target cancer or virally-infected cells. Repeated dosing with Tarmogens further increases the number of T cells available to eliminate diseased cells. In summary, Tarmogens couple the innate immune response to yeast with a potent activation of antigen-specific cellular immune responses against cancer cells or virally infected cells.

For more information, visit www.globeimmune.com

Abstract

TRANSCRATIONAL PATTERNS OF PANCREAS CANCER RAS MUTATIONS AND DISCOVERY OF A NEW MUTATION WITH ONCOCENE SYNERGY WHEN FOUND WITH RAS COBON 12 MUTATIONS

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Background:

Patterns of KRas oncprotein mutations at amino acids 12, 13, or 61 drive uncontrolled cell proliferation and tumorigenesis. We have developed yeast-based immunotherapy targeting Ras point mutations (G12V or G12A, Q61R, Q61H, E76G or E76K) and we analyzed the incidence of specific Ras point mutations among patients with pancreatic cancer in Bulgaria, India, Taiwan and the US.

Experimental procedures:

Tumor biopsies were obtained from 222 patients in Bulgaria, India, Taiwan and the US. DNA sequencing was characterized for tumor-associated mutations in K-ras exons 2 and 3 by nested PCR amplification including pyrosequencing and RFLP scanning. Single G12V, Q61R, Q61H or E76K mutations or double G12V-Q61R, Q61R-E76K mutations were encoded into the mouse K-ras gene than transduced into BALB/c-3T3 fibroblast cells. The transfected cells were seeded for growth in soft agar or implanted into BALB/c athymic nude mice for tumorigenesis. The immediate outcome of GTP-bound Ras protein, a marker of Ras activation, was assayed with cells transfected to express extra copies of wild type Ras, or Ras with single or double mutations.

Results:

Notable differences from previously reported patterns included i) a different profile of Ras mutation frequencies found in pancreatic cancer in different countries; ii) the unanticipated frequency of amino acid 61 Ras mutations (Ras Q61H); and iii) the discovery of a new Ras mutation at amino acid 76 (Ras E76G or E76K) that was detected in patient tumors as a single mutation or simultaneously with amino acid 12 or 61 mutations.

The role of Ras oncprotein-bearing 67% mutations alone or in combination with G12 mutations in tumorigenesis was investigated in preclinical models. Cells transfected with K-ras gene encoding single G12V, Q61R or Q61H mutations or double G12V-Q61R, Q61R-E76K mutations or double G12V-Q61R, Q61R-E76K mutations all exhibited the transformed phenotype by forming colonies in soft agar. Cells expressing single mutant Ras had increased intracellular levels of GTP-bound Ras protein compared to overexpression of wild type Ras protein and GTP-bound Ras levels were further amplified in cells expressing Ras with double G12V-E76K mutations. To confirm tumorigenesis in vivo, tumors originating from double G12V-E76K mutations implanted into BALB/c mice showed more aggressive growth rates compared to those expressing any single mutant Ras.

Conclusions:

Tumor genotyping reveals differences in transnational incidence of pancreatic cancer Ras mutations and discoveries of a new Ras mutation with oncogene synergy when found with Ras codon 12 mutations.


