INTRODUCTION

Heterotrimeric G proteins regulate a multitude of signaling pathways by providing signal-coupling mechanisms to seven transmembrane receptors (1). Of the four different classes of G proteins, G12 class, defined by the α-subunits Gα12 and Gα13, is primarily involved in pathways regulating cell growth and development (2). In this context, it is of interest to note that Gα12 was identified as a potential oncogene during a search for the causative onco-

ORIGINAL ARTICLE

Proliferation-Specific Genes Activated by Gα12

A Role for PDGFRα and JAK3 in Gα12-Mediated Cell Proliferation

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Abstract

Gα12, the α-subunit of G protein G12, is ubiquitously expressed and it has been identified as a putative “causative oncogene” of soft-tissue sarcomas. Overexpression of wild-type or GTPase-deficient mutant of Gα12 (Gα12Q229L or Gα12QL) leads to the oncogenic transformation of NIH3T3 cells. Gα12QL-transformed NIH3T3 cells show a distinct oncogenic phenotype defined by increased cell proliferation, anchorage-independent growth, reduced growth-factor dependency, attenuation of apoptotic signals, and neoplastic cytoskeletal changes. In this study, the genes contributing to the reduced growth-factor dependency of Gα12QL–NIH3T3 cells were identified by transcription profiling of serum-starved Gα12QL-transformed NIH3T3 (Gα12QL–NIH3T3) cells. Results from these studies indicate that Gα12QL stimulates the expression of genes that promote cell growth. The increased expressions of growth-promoting genes in Gα12QL–NIH3T3 cells were validated by semiquantitative reverse transcription–polymerase chain reaction and immunoblot analyses. Further studies aimed at investigating the critical role of two of such upregulated genes, namely PDGFRα and JAK3, indicated that the inhibition of PDGFRα or JAK3 activity-attenuated Gα12QL-mediated serum-independent cell proliferation. These studies point to possible novel autocrine and/or paracrine control mechanisms involving PDGFRα and JAK3 in Gα12-mediated proliferation and oncogenesis.

Index Entries: G Protein; PDGFR; JAK3; oncogene; cell transformation; cell proliferation.

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gene of soft-tissue sarcoma (3). Consistent with this observation, expression of GTPase-deficient constitutively active mutant of Gα12 (Gα12Q229L or Gα12QL) has been shown to induce oncogenic transformation of fibroblast cell lines (4–6). Gα12QL-transformed NIH3T3 cells exhibit “typical” oncogenic phenotypes, namely (1) reduced growth-factor dependency, (2) increased cell proliferation, (3) anchorage-independent growth, (4) attenuation of apoptotic signals, and (4) neoplastic cytoskeletal changes. Previous studies have indicated that the signaling pathways regulated by Gα12 involve a diverse set of downstream signaling pathways involving small GTPases as well as Ser/Thr kinases (2). In addition, a role for tyrosine kinase ETK as well as β-catenin in Gα12-mediated signaling pathways has been demonstrated (7,8). However, the causative signaling factors involved in the epigenetic changes leading to Gα12-mediated oncogenic transformation and the relative contribution of these different factors in Gα12-mediated oncogenic transformation are largely unknown.

To identify the genes that contribute to Gα12QL-mediated reduced growth-factor dependency, transcriptional profiling of serum-starved Gα12QL-transformed NIH3T3 (Gα12QL–NIH3T3) cells was carried out and compared with those of the vector control cells. Our results presented here from such analysis indicate that Gα12-mediated serum-independent growth involves the upregulation of several unique sets of genes, including those of growth factors, growth-factor receptors, signal-transducers, and transcription factors. These studies identify platelet-derived growth receptor α (PDGFRα) and Janus kinase-3 (JAK3) as two such candidate genes that confer the reduced growth-factor dependency of Gα12QL–NIH3T3 transformants. Consistent with this finding, treatment of cells with a PDGFR-specific or JAK3-specific inhibitor potently inhibits the serum-independent proliferation of Gα12QL–NIH3T3 cells. In addition to identifying the critical genes involved in Gα12-mediated oncogenic transformation, our studies presented here demonstrate, for the first time, that Gα12-mediated upregulation of PDGFRα and JAK3 are involved in conferring one of the hallmarks of oncogenic phenotype, namely the reduced growth-factor dependency of Gα12QL transformants.

MATERIALS AND METHODS

Cell Culture

NIH3T3 cells were maintained by serial passage in Dulbecco’s modified Eagle’s medium (DMEM; Cellgro, VA) containing 5% calf serum (Life Technologies, Inc.), 50 U/mL penicillin, and 50 µg/mL streptomycin at 37°C in a 5% CO2 incubator. Serum deprivation of NIH3T3 cells was done by incubation for 24 h in DMEM supplemented with 10 mM HEPES (pH 7.4) and 0.2% bovine serum albumin (BSA). The pcDNA3–NIH3T3 and Gα12Q229L–NIH3T3 cell lines have already been described (9). For serum stimulation of vector-control and Gα12QL–NIH3T3 cells, either 4 × 10^5 cells (60-mm tissue culture plate) or 1 × 10^6 cells (100-mm tissue culture plate) were plated and allowed to grow for 24 h. At this point, the cells were serum-starved for 24 h and then subjected to serum stimulation for different lengths of time.

RNA Extraction and cDNA Synthesis

Total RNA from cells was isolated using the guanidinium thiocyanate (GTC) method and an aliquot of total RNA (5 µg) was taken and cDNA probes were synthesized according the manufacturer’s (BD Biosciences Clontech) protocol.

Microarray Screening and Analysis

We used the Atlas Mouse cDNA expression cDNA array (BD Biosciences Clontech, CA; cat. no. 7741-1), which contains a duplicate set of 588 known genes and 21 housekeeping genes. A list of the arrayed genes with GenBank accession numbers is available (http://www.clontech.com/atlas/genelists/index.shtml). The hybridization of cDNA