Isolation of a Novel Candidate Oncogene within a Frequently Amplified Region at 3q26 in Ovarian Cancer

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ABSTRACT

Amplification of 3q25-q26 was one of the most frequent chromosomal alterations in human ovarian carcinoma. A chromosome microdissection-hybrid selection method was applied to isolate transcribed sequences from a primary ovarian cancer containing high-copy-number amplification of 3q26 using 3q26 band-specific DNAs generated by chromosome microdissection. Using this method, we have isolated a novel candidate oncogene eIF-5A2 (eukaryotic initiation factor 5A2). eIF-5A2 shares 82% identity of amino acid sequence with eIF-5A including the minimum domain needed for eIF-5A maturation by hypusination modification at lysine-50 residue. Amplification and overexpression of eIF-5A2 was frequently detected in primary ovarian cancers and ovarian cancer cell lines. The proliferation-related function of eIF-5A2 suggests that eIF-5A2 is a candidate oncogene related to the development of ovarian cancer.

INTRODUCTION

Ovarian cancer is the leading cause of death from female gynecological cancers and will affect approximately 1% of American women during their lifetime (1). The prognosis of ovarian cancer is very poor, with a 5-year survival rate of <20% if not diagnosed early. Compared with other solid tumors, relatively little is known regarding the molecular pathogenesis and progression of ovarian cancer. Recently, a frequently amplified region at 3q25-qter was identified in primary ovarian carcinoma by CGH (2-4). The minimum overlapping amplified region has been mapped to 3q26. In our CGH study, gain of 3q was detected in 15 of 30 (50%) primary ovarian cancers. High-copy-number amplification of 3q within a minimal overlapping region at 3q26 was observed in four cases (OV-4, OV-7, OV-13, and OV-27). Gain of 3q has also been frequently detected in other solid tumors including esophageal carcinoma (5), prostate cancer (6), and carcinoma of the fallopian tube (7). These studies strongly indicate that 3q26 may contain an oncogene(s) related to the pathogenesis and progression of various solid tumors, including ovarian cancer.

Identification of commonly amplified chromosomal regions and corresponding oncogenes within the region is imperative to understand the molecular mechanism of tumorigenesis. Recently, PIK3CA which encodes a catalytic subunit of phosphatidylinositol 3-kinase has been implicated as a candidate oncogene at 3q26 (8). Since only low-level amplification of PIK3CA was detected in OV-4, OV-27, and an ovarian cancer cell line UACC-1598 that contains high-level amplification of 3q26 within a minimal overlapping region at 3q26 may contain an oncogene(s) related to the pathogenesis and molecular progression of various solid tumors including esophageal carcinoma (5), prostate cancer (6), and carcinoma of the fallopian tube (7). These studies strongly indicate that 3q26 may contain an oncogene(s) related to the pathogenesis and progression of various solid tumors, including ovarian cancer.

RESULTS

Chromosome Microdissection. To identify the candidate oncogene(s) within the 3q26-amplified region, chromosome microdissec-
was performed on normal ovary tissue and tissues from liver, placenta, breast, skin, brain, and colon. The results showed that eIF-5A2 is expressed in all of these tissues (Fig. 3C). Expressed sequence tag database search through BLAST revealed that eIF-5A2 is also expressed in human testis, uterus, lymphocyte, and lung tissues.

**Map of eIF-5A2 to 3q26.2.** To confirm the chromosome localization, a BAC clone containing the eIF-5A2 sequence was isolated through BLAST searching. The BAC clone was confirmed containing eIF-5A2 by Southern blot analysis (Fig. 3A) and PCR using designed primers within an exon (204 bp; Fig. 3B). This BAC clone was then mapped to 3q26.2 by FISH (Fig. 1, C and D).

**Amplification of eIF-5A2 in Ovarian Cancer.** DNA sequence amplification of eIF-5A2 in 30 primary ovarian cancers was studied by Southern blot analysis. Amplification of eIF-5A2 was detected in

Fig. 1. A, amplification of 3q25-q26 in two primary ovarian cancers detected by CGH. Tumor DNA and normal reference DNA was labeled with green and red color, respectively. B, microdissected DNA from 3q26 (red) was hybridized to normal metaphase chromosomes (partial metaphase was shown). C, G-banded partial metaphase with arrow to region of hybridization at 3q26.2. D, identical partial metaphase as c after FISH with BAC clone (red) containing eIF-5A2, which was localized at 3q26.2.

Fig. 2. Sequence alignment of the human eIF-5A2-deduced amino acid sequence with human eIF-5A and chicken eIF-5A; those identical sequences are highlighted in blue. The minimum domain of eIF-5A needed for hypusine modification is boxed. * lysine residue for posttranslationally modified to hypusine.

Fig. 3. The BAC clone containing eIF-5A2 gene was confirmed both by Southern blot analysis (A) and by PCR using designed primers within an exon (204 bp; B). The genomic DNAs prepared from normal placenta and the BAC clone were digested with EcoR I and hybridized with eIF-5A2 cDNA. C, expression of eIF-5A2 in six normal tissues was detected using RT-PCR.

Fig. 4. A, Southern blot analysis demonstrates amplification of eIF-5A2 in five primary ovarian cancers and the ovarian cancer cell line UACC-1598. Fifteen micrograms of EcoR I-digested DNAs were separated by electrophoresis in 0.8% agarose gel and hybridized with a 1.2-kb eIF-5A2 cDNA probe. A probe for β-actin was used for loading control. B, Northern blot analysis of eIF-5A2 in four primary ovarian cancers and UACC-1598. Ten micrograms of total RNA were loaded in each lane. The Northern blot was hybridized with a 1.2-kb eIF-5A2 cDNA probe. The RNA separating gel stained with ethidium bromide for the Northern blot was used as loading control.
15 of 30 cases and high-copy-number amplification was observed in 4 cases (OV-4, OV-7, OV-13, and OV-27; Fig. 4A). High-copy-number amplification of eIF-5A2 was also detected in the ovarian cancer cell line UACC-1598, which contains 3q26 amplification in the form of double minutes (Fig. 4A). Expression level of eIF-5A2 in four primary ovarian cancers (OV-4, OV-7, OV-24, and OV-27) and ovarian cancer cell line UACC-1598 was analyzed by Northern blot and overexpression of eIF-5A2 was observed in all cases (Fig. 4B). To confirm whether the double minutes contain eIF-5A2, metaphase prepared from UACC-1598 was hybridized with the BAC clone containing eIF-5A2 by FISH. The result demonstrated that the BAC clone hybridized to all double minutes (Fig. 5A). Amplification of eIF-5A2 was also observed in another ovarian cancer cell line (OVCAR3) by FISH (Fig. 5B). According to our CGH results, a minimal overlapping amplicon at 3q26.1-26.2 was defined and eIF-5A2 is mapped exactly within the region (Fig. 5C).

**DISCUSSION**

We describe in this report the application of a chromosome microdissection-hybrid selection technique to isolate a novel member of the eIF-5A family, eIF-5A2, from a frequent amplified region at 3q26 in ovarian cancer. The identity of the amino acid sequence between eIF-5A2 and eIF-5A is 82%, including the minimum domain needed for hypusine modification and the lysine-50 residue where the hypusine residue can be formed by posttranslational modification. This indicates that eIF-5A2 is an eIF-5A family member and may have similar function.

It has been suggested that eIF-5A plays a role in the translation initiation; however, the initiation of protein synthesis in eIF-5A-depleted yeast cells was not significantly affected (14). Although the precise function of eIF-5A is unclear, the necessity for the hypusine-containing eIF-5A for cell proliferation is well studied (14, 15). It has been demonstrated that complete intracellular depletion of eIF-5A by gene deletion resulted in inhibition of cell growth (13). Other studies indicated that the inhibition of deoxyhypusine synthase, the enzyme involved in the hypusination reaction of eIF-5A, inhibited Chinese hamster ovary cell proliferation (16), suppressed the growth of HeLa cells and v-src-transformed NIH3T3 cells (17). Hanauske-Abel et al. (18) suggested that hypusine containing eIF-5A may directly affect the expression of a selective set of genes involved in the G1 to S transition of the cell cycle in the eukaryotic cells since the cell cycle could be arrested at the G1-S boundary by inhibition of eIF-5A.
hypusine modification. Recently, Tome et al. (19) reported that suppression of eIF-5A hypusine modification by excess putrescine accumulation or treated cells with diaminohexane is one mechanism by which cells may be induced to undergo apoptosis. A recent study showed that the hypusine formation activity is serum responsive and significantly increased by >30-fold in Ras oncogene-transformed NIH3T3 cells (20). All of these studies strongly suggested the association between eIF-5A and cancer development.

In the present study, amplification of eIF-5A2 was detected in 15 of 30 primary ovarian cancers and several ovarian cancer cell lines, including UACC-1598 which contains high-copy-number amplification of the 3q26 region. Overexpression of eIF-5A2 was observed in all four tested primary ovarian cancers with 3q26 amplification. In addition, a BAC clone containing eIF-5A2 was hybridized to double minutes in the ovarian cancer cell line UACC-1598. Therefore, based on its chromosomal location, its amplification status in ovarian cancer, and its possible proliferation-related function, eIF-5A2 is considered as the putative oncogene within the minimal overlapping amplicom at 3q26.2 in ovarian cancer.

The amplified region of 3q26 may contain more than one important gene, including PIK3CA and eIF-5A2 which are the biological targets of amplification events in ovarian cancer. eIF-5A2 is telomeric to PIK3CA and the genomic distance between them is about 7cM. Further studies of the amplification and expression of eIF-5A2 in a large set of ovarian cancers using tissue microarray as well as determination of its function will be necessary to address the relationship between eIF-5A2 and ovarian cancer.

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REFERENCES

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