

## Malignant Transformation and Abnormal Expression of Eukaryotic Initiation Factor in Bronchial Epithelial Cells Induced by Cadmium Chloride<sup>1</sup>

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**Objective** To analyze the relationship between malignant transformation and abnormal expression of eukaryotic initiation factor 3 (eIF3 p36) in human bronchial epithelial (16HBE) cells induced by cadmium chloride (CdCl<sub>2</sub>). **Methods** 16HBE cells were treated several times with different concentrations of CdCl<sub>2</sub>. Tumorigenic potential of transformed cells was identified by assays for anchorage-independent growth in soft agar and for tumorigenicity in nude mice after the 35th passage. Total RNA was isolated from 16HBE cells induced by CdCl<sub>2</sub>, including non-transformed, Cd-transformed, and Cd-tumorigenic cell lines. Special primers for eIF3 p36 were designed and the expression of eIF3 mRNA in different cell lines was detected with fluorescent quantitative-polymerase chain reaction technique (FQ-PCR). **Results** The 35th passage of 16HBE cells transformed by CdCl<sub>2</sub> exhibited overlapping growth. Compared with the non-transformed cells, colonies of transformed cell lines in soft agar showed statistically significant increases and dose-dependent effects ( $P < 0.01$ ). All Cd-induced transformed cell lines formed tumors in nude mice within 2 weeks of inoculation, but none of the mice injected with non-transformed cells showed tumors even after 3 weeks. All tumors were pathologically identified as poorly differentiated squamous cell carcinoma. The eIF3 p36 genes in different stages of 16HBE cells transformed by CdCl<sub>2</sub> were elevated as compared with the non-transformed control ( $P < 0.01$ ), and the eIF3 expression increased with the degree of cell malignancy. **Conclusion** CdCl<sub>2</sub> is capable of inducing morphological transformation in 16HBE cells and transformed cells are potentially tumorigenic. Over-expression of eIF3 p36 is positively correlated with malignant transformation of 16HBE cells induced by CdCl<sub>2</sub> and may be one of the molecular mechanisms potentially responsible for carcinogenesis due to Cd.

**Key words:** Cell transformation; Tumorigenicity; Eukaryotic initiation factor 3; Cadmium chloride; Human bronchial epithelial cells

### REFERENCES

- Fay R M, Mumtaz M M (1996). Development of a priority list of chemical mixture occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem Toxicol* **34**, 1163-1165.
- Waalkes M P (2003). Cadmium carcinogenesis. *Mutat Res* **533**(1-2), 107-120.
- Sorahan T, Esmen N A (2004). Lung cancer mortality in UK nickel-cadmium battery workers, 1947-2000. *Occup Environ Med* **61**(2), 108-116.
- Koyu A, Gokcimen A, Ozguner F, et al. (2006). Evaluation of the effects of cadmium on rat liver. *Mol Cell Biochem* **20**, 1-5.
- Nadal M, Schuhmacher M, Domingo J L (2004). Metal pollution of soils and vegetation in an area with petrochemical industry. *Sci Total Environ* **321**(1-3), 59-69.
- Boguszewska A, Pasternak K (2004). Cadmium--influence on biochemical processes of the human organism. *Ann Univ Mariae Curie Sklodowska* **59**(2), 519-523.
- Sunderman F M (2001). Nasal toxicity, carcinogenicity, and olfactory uptake of metals. *Ann Clin Lab Sci* **31**, 3-24.
- Nordberg G, ChinaCad Group (2003). Cadmium and human health: A perspective based on recent studies in China. *J Trace Elem Exp Med* **16**, 307-319.
- Kriegel A M, Soliman A S, Zhang Q, et al. (2006). Serum cadmium levels in pancreatic cancer patients from the East Nile Delta region of Egypt. *Environ Health Perspect* **114**(1), 113-119.
- Sorahan T (1987). Mortality from lung cancer among a cohort of nickel cadmium battery workers: 1946-84. *Br J Ind Med* **44**, 803-809.
- Goyer R A, Liu J, Waalkes M P (2004). Cadmium and cancer of prostate and testis. *Biometals* **17**(5), 555-558.
- IARC (1993): Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, Vol. 58: 119-238, International Agency for Research on Cancer, Lyon, France.
- Joseph P, Lei Y X, Whong W Z, et al. (2002). Molecular Cloning and Functional Analysis of a Novel Cadmium-responsive Proto-oncogene. *Cancer Res* **62**, 703-707.
- Lei Y X, Joseph P, Ong T M (2002). Antisense inhibition of

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- translation initiation factor 3 reverse its oncogenic potential. *Teratog Carcinog Mutagen* **22**(6), 403-409.
15. Keshava N, Zhou G, Hubbs A F, *et al.* (2000). Transforming and carcinogenic potential of cadmium chloride in BALB/c-3T3 cells. *Mutat Res* **448**(1), 23-28.
  16. Cozens A L, Yezzi M J, Kunzelmann K, *et al.* (1994). CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* **10**(1), 38-47.
  17. Dunkel V C, Rogers C, Swiernga S H, *et al.* (1991). Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: III. Cell transformation in C3H/10T1/2 mouse embryo cell, BALB/c-3T3 mouse fibroblast and Syrian hamster embryo cell cultures. *Mutat Res* **246**, 285-300.
  18. Nanni P, Pupa S M, Nicoletti G, *et al.* (2000). p185 neu protein is required for tumor and anchorage-independent growth, not for cell proliferation of transgenic mammary carcinoma. *Int J Cancer* **87**, 186-194.
  19. Gelmini S, Orlando C, Sestini R, *et al.* (1997). Quantitative polymerase chain reaction-based homogeneous assay with fluorogenic probes to measure c-erbB-2 oncogene amplification. *Clin Chem* **43**(5), 752-758.
  20. Barrett J C (1980). A preneoplastic stage in the spontaneous neoplastic transformation of Syrian hamster embryo cell culture. *Cancer Res* **40**, 91-94.
  21. Berwald Y, Sachs L (1963). *In vitro* transformation with chemical carcinogens. *Nature* **200**, 1182-1184.
  22. Berwald Y, Sachs L (1965). *In vitro* transformation of normal cells to tumor cells by carcinogenic hydrocarbons. *J Natl Cancer Inst* **35**, 641-661.
  23. Saffiotti U, Bertolero F (1989). Neoplastic transformation of BALB/3T3 cells by metals and quest for induction of a metastatic phenotype. *Biol Trace Elem Res* **21**, 475-482.
  24. Landolph J R (1985). Mechanisms of chemically induced multistep neoplastic transformation in C3H10T1/2 cells. *Carcinogenesis* **10**, 211-223.
  25. Cairns J (1975). Mutation selection and the natural history of cancer. *Nature* **255**, 197-200.
  26. Cruenert D C (1995). Finkbeiner W E, Widdicombe J H: Culture and transformation of human airway epithelial cells. *Am J Physiol* **268**, 1347-1360.
  27. Vogelstein B, Kinzler R W (1993). The multistep nature of cancer. *Trends Genet* **9**, 138-141.
  28. Zimmer S G, Debenedetti A, Graff J R (2000). Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor promotion, growth, invasion, and metastasis. *Anticancer Res* **20**, 1343-1352.
  29. Riis B, Rattan S I S, Clark B F C, *et al.* (1990). Eukaryotic translation elongation factors. *TIBS* **15**, 420-424.
  30. Sonenbeerg N (1990). Translation factors as effectors of cell growth and tumorigenesis. *Curr Opin Cell Biol* **5**, 955-960.
  31. Nupponen N N, Porkka K, Kakkola L, *et al.* (1999). Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer. *Am J Pathol* **154**(6), 1777-1783.
  32. Mayeur G L, Hershey J W (2002). Malignant transformation by the eukaryotic translation initiation factor 3 subunit p48 (eIF3e). *FEBS Lett* **514**(1), 49-54.

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