

【S-1】

Inactivation of SMAD4 Tumor Suppressor gene during Gastric Cancer Progression

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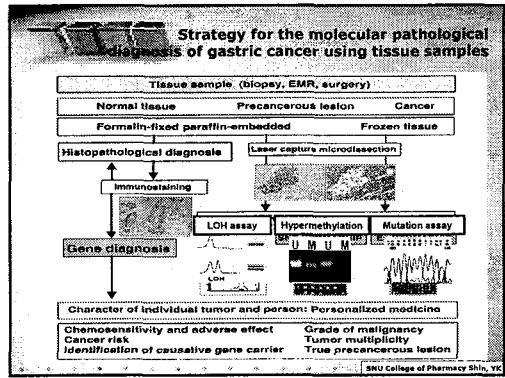
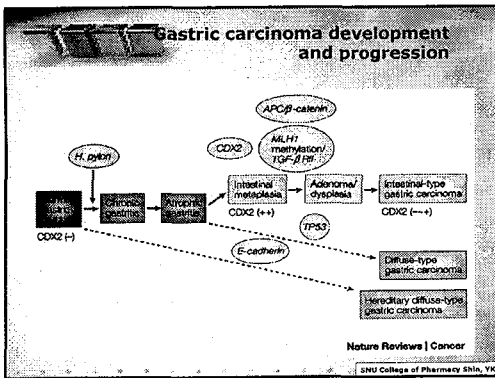
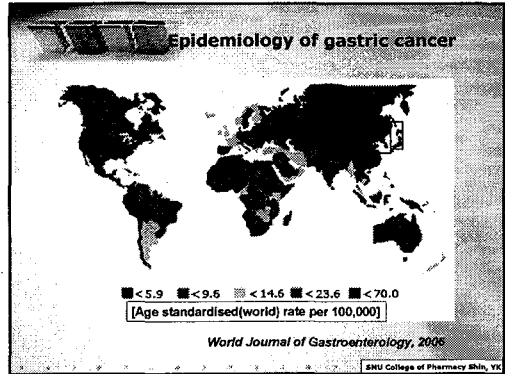
Mothers against decapentaplegic homolog 4 (SMAD4) is a tumor suppressor gene associated with gastrointestinal carcinogenesis. The aim of the present study was to characterize more precisely its role in the development and progression of human gastric carcinoma.

In this study, using tissue microarray analysis of 283 gastric cancers and related lesions, we found loss of SMAD4 protein expression in the cytoplasm (36/114, 32%) and in the nucleus (46/114, 40%) of gastric cancer cells. The loss of nuclear SMAD4 expression in primary tumors correlated significantly with poor survival, and was an independent prognostic marker in multivariate analysis. We also found a substantial decrease in SMAD4 expression at both the RNA and protein level in several human gastric carcinoma cell lines. To identify the genetic and/or epigenetic mechanisms of altered SMAD4 expression in gastric carcinoma, loss of heterozygosity (LOH), promoter hypermethylation, and exon mutations were examined. We found that LOH (20/70, 29%) and promoter hypermethylation (4/73, 5%) were associated with the loss of SMAD4 expression. SMAD4 protein levels were also affected in certain gastric carcinoma cell lines following incubation with MG132, a proteasome inhibitor.

Taken together, our results indicate that the loss of SMAD4, especially loss of nuclear SMAD4 expression, is involved in gastric cancer progression. The loss of SMAD4 in gastric carcinomas is due to several mechanisms, including LOH, hypermethylation, and proteasome degradation.

Smad4 in gastric carcinogenesis

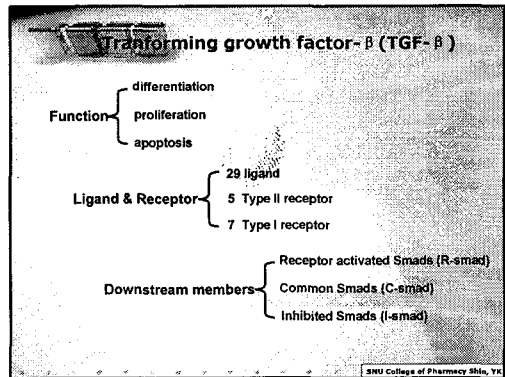
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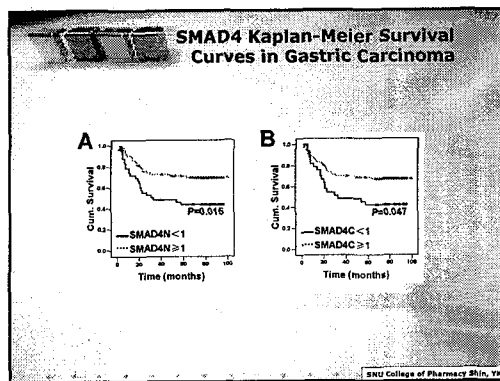
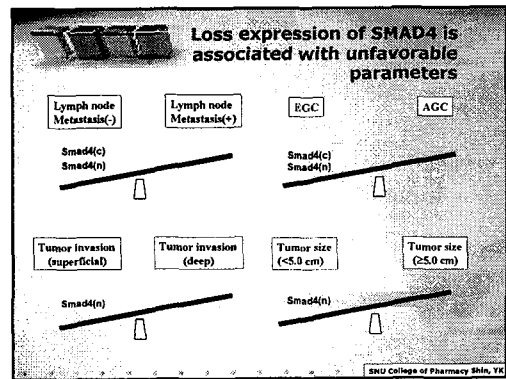
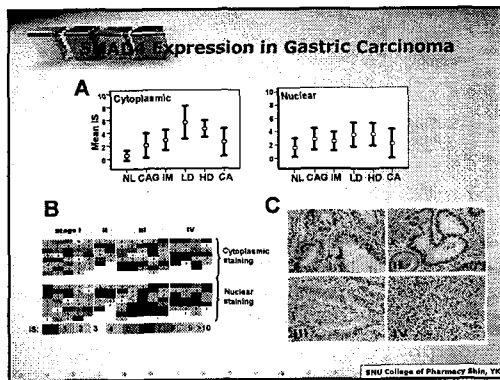
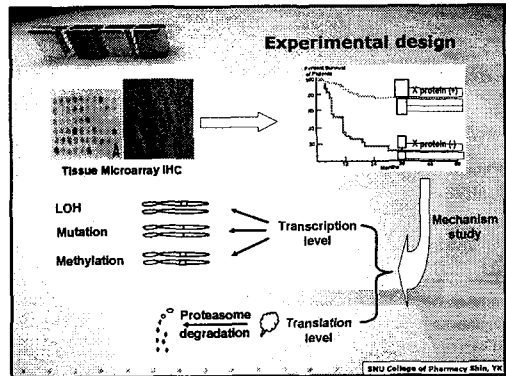
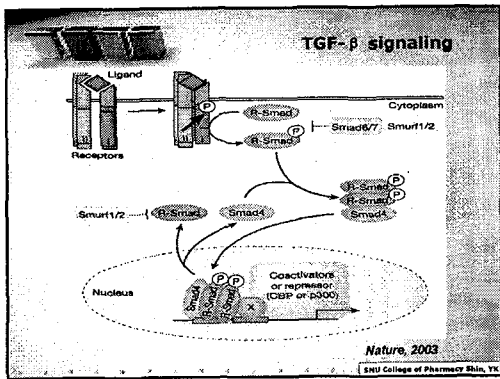


Genetic alterations of gastric cancers

| | Diffuse type(%) | Intestinal type(%) |
|--|-----------------|--------------------|
| Oncogene activation | | |
| <i>f-catenin</i> | 0 | 17-27 |
| <i>K-ras</i> | 0-6 | 6-16 |
| <i>C-myc</i> | 12-13 | 12-13 |
| Inactivation of tumor suppressor gene | | |
| <i>p53</i> | 0-21 | 34-42 |
| <i>APC</i> | 0-5 | 5 |
| <i>E-cadherin</i> | 32-50 | 5 |
| <i>p16</i> | 0 | 5 |
| <i>p18</i> | 11 | 35 |
| Methylation | | |
| Microsatellite instability | 5-22 | 13-41 |

Cancer cell, 2004



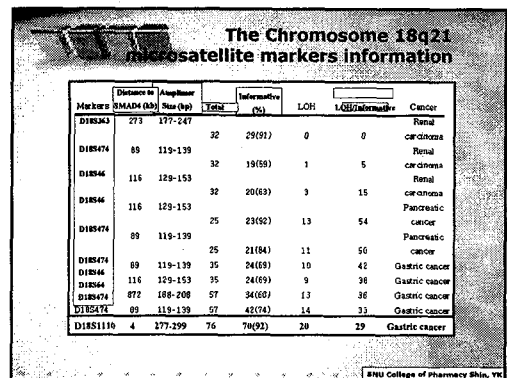
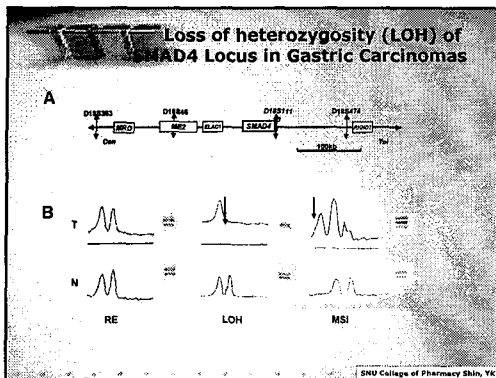
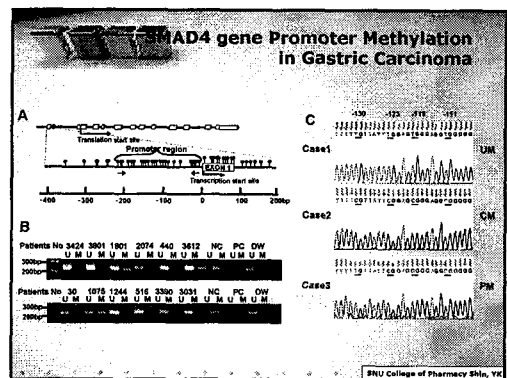
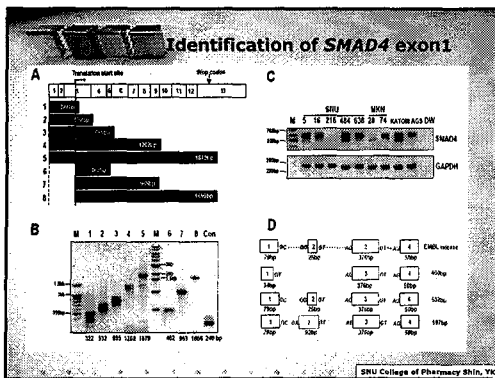
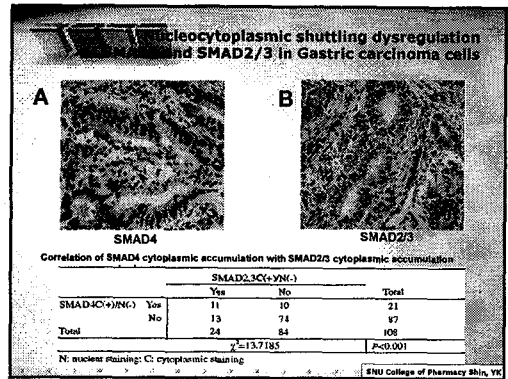
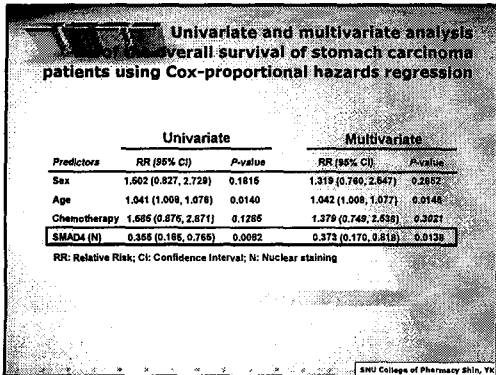


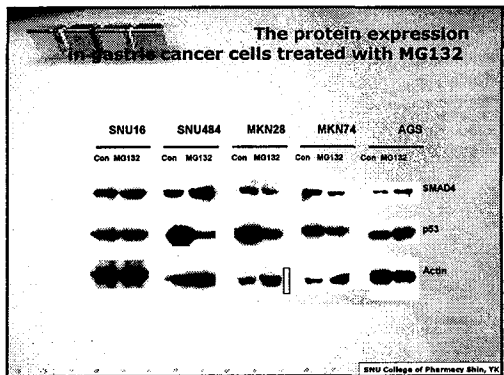
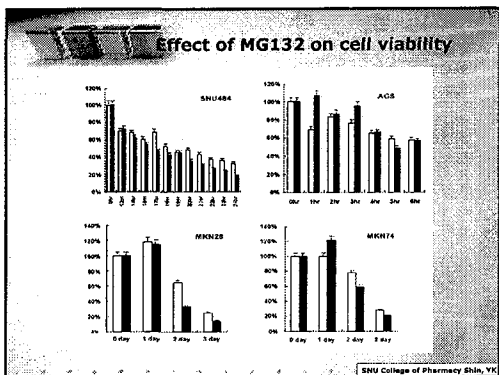
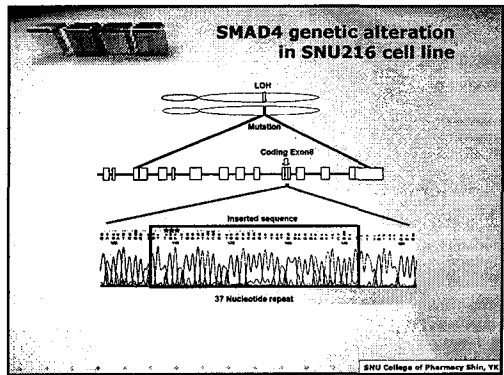
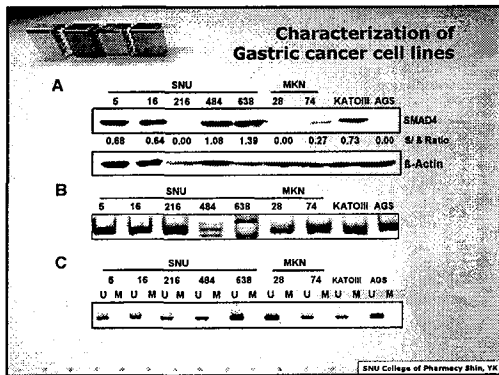
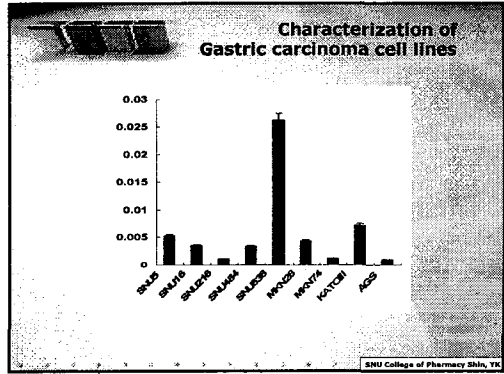
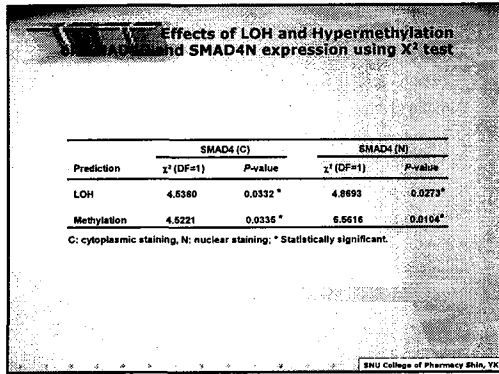
Univariate analysis of the overall survival of gastric cancer patients using Kaplan-Meier survival analysis

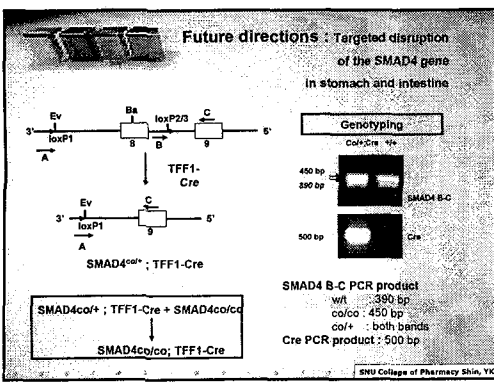
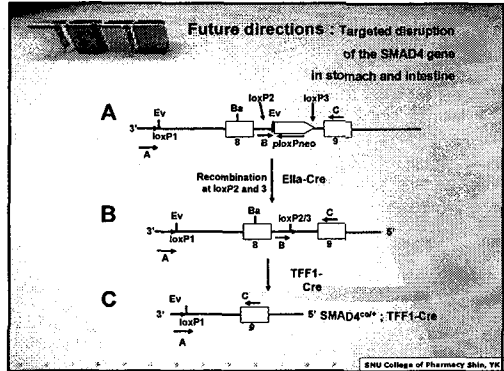
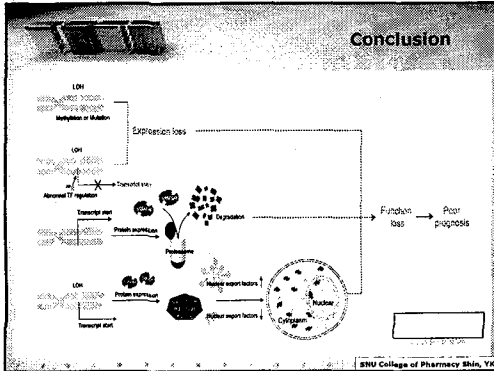
| Variables | No. of patients | Overall survival (%) | P-value |
|-------------------|-----------------|----------------------|---------|
| Age | | | |
| < 60 | 24 | 52.36 | 0.008 |
| ≥ 60 | 40 | 45.00 | 0.176 |
| Sex | | | |
| M | 54 | 51.13 | 0.822 |
| F | 40 | 48.00 | 0.723 |
| Tumor stage | | | |
| I-II | 53 | 88.04 | 98.04 |
| III | 26 | 66.15 | 32.73 |
| IV | 26 | 37.69 | 0.001* |
| Tumor size | | | |
| ≤ 5.0 | 40 | 66.67 | 95.10 |
| > 5.0 | 65 | 61.08 | 30.17 |
| 0.0004* | | | |
| Depth of invasion | | | |
| T1 | 82 | 74.05 | 91.03 |
| T2 | 78 | 69.00 | 18.00 |
| 0.001* | | | |
| ECG | | | |
| Type I | 43 | 67.43 | 85.14 |
| Type II | 78 | 70.26 | 52.76 |
| 0.0093* | | | |
| Chemotherapy | | | |
| No | 67 | 64.87 | 61.83 |
| Yes | 5 | 83.33 | 64.00 |
| 0.578 | | | |
| SMAD4(c/n) | | | |
| c | 45 | 62.83 | 74.83 |
| n | 3 | 100 | 100 |
| 0.047* | | | |
| SMAD4(n) | | | |
| < 1 | 34 | 59.56 | 52.76 |
| ≥ 1 | 78 | 65.74 | 48.96 |
| 0.004* | | | |
| TP53 | | | |
| WT | 66 | 63.00 | 73.89 |
| M | 20 | 55.50 | 60.00 |
| 0.910 | | | |
| HER2/neu | | | |
| - | 79 | 79.74 | 60.64 |
| + | 2 | 0.00 | 0.00 |
| 0.723 | | | |

T1: submucosal invasion; T2: up to gastric muscle; N: nodal invasion; ECG: lymphatic invasion; C: cytoplasmic staining; n: nuclear staining.

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