

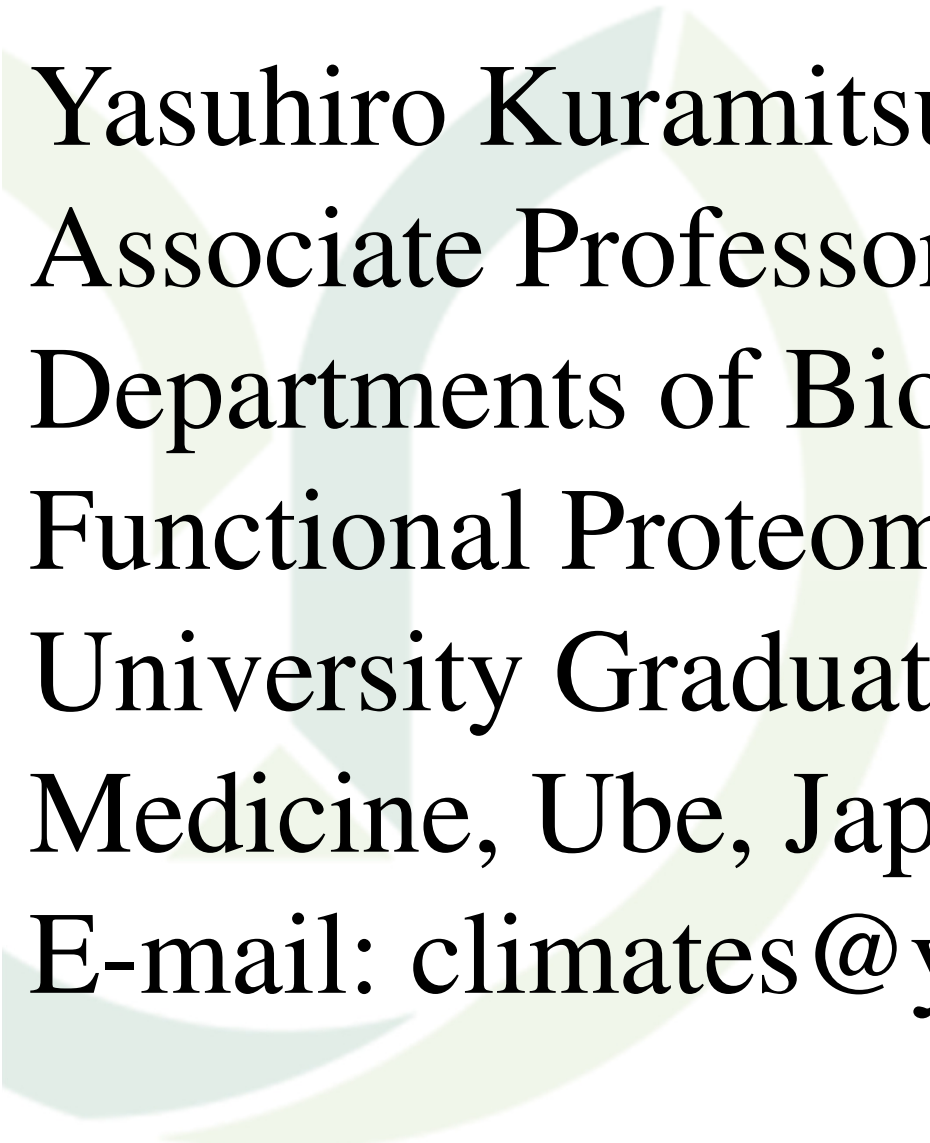
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Pancreatic cancer remains one of the diseases with poor prognosis, and the most patients have no chance to be operated when they are diagnosed as pancreatic cancer. Their 5-year survival rate is less than 5%. Gemcitabine is one of the most effective chemotherapeutic drugs for pancreatic cancer, but the therapeutic effect is insufficient because many types of pancreatic cancer are resistant. We have reported that *HSP27 was one of the important factors related to gemcitabine-resistance*. We treated gemcitabine-resistant pancreatic cancer cells with interferon-gamma or KNK-437, which were reported as HSP27-suppressors, and showed the suppressive effects of HSP27 and combinatorial effects on inhibition of proliferation against gemcitabine-resistant pancreatic cancer cells.

AHCC is an extract of basidiomycete mushroom and includes polysaccharide, and has been used as health food to enhance the therapeutic effects and reduce the adverse effects of chemotherapy. In the present study we *investigated the effects of AHCC on the expression of HSP27, and the effects of combinatorial treatment of AHCC and gemcitabine on the gemcitabine-resistant pancreatic cancer cells*, and **AHCC down-regulated HSP27 and showed an anti-proliferative effect on gemcitabine-resistant cells.**

Prognosis of pancreatic cancer

JPS-stage at the time of diagnosis

| Stage | n | 5 year survival rate |
|-----------|------|----------------------|
| Stage I | 128 | 58.6% |
| Stage II | 192 | 51.0% |
| Stage III | 1039 | 25.9% |
| Stage IVa | 1809 | 11.9% |
| Stage IVb | 4661 | 2.8% |

Therapy and prognosis

| Method of therapy | 5 year survival rate |
|----------------------|----------------------|
| Resection | 12.2% |
| Palliative operation | 0% |
| Laparotomy | 2.0% |
| Non-operation | 0% |

SUIZOU 2003 (Japanese).

Gemcitabine (gemzar, GEM), a drug used for chemotherapy against pancreatic cancer

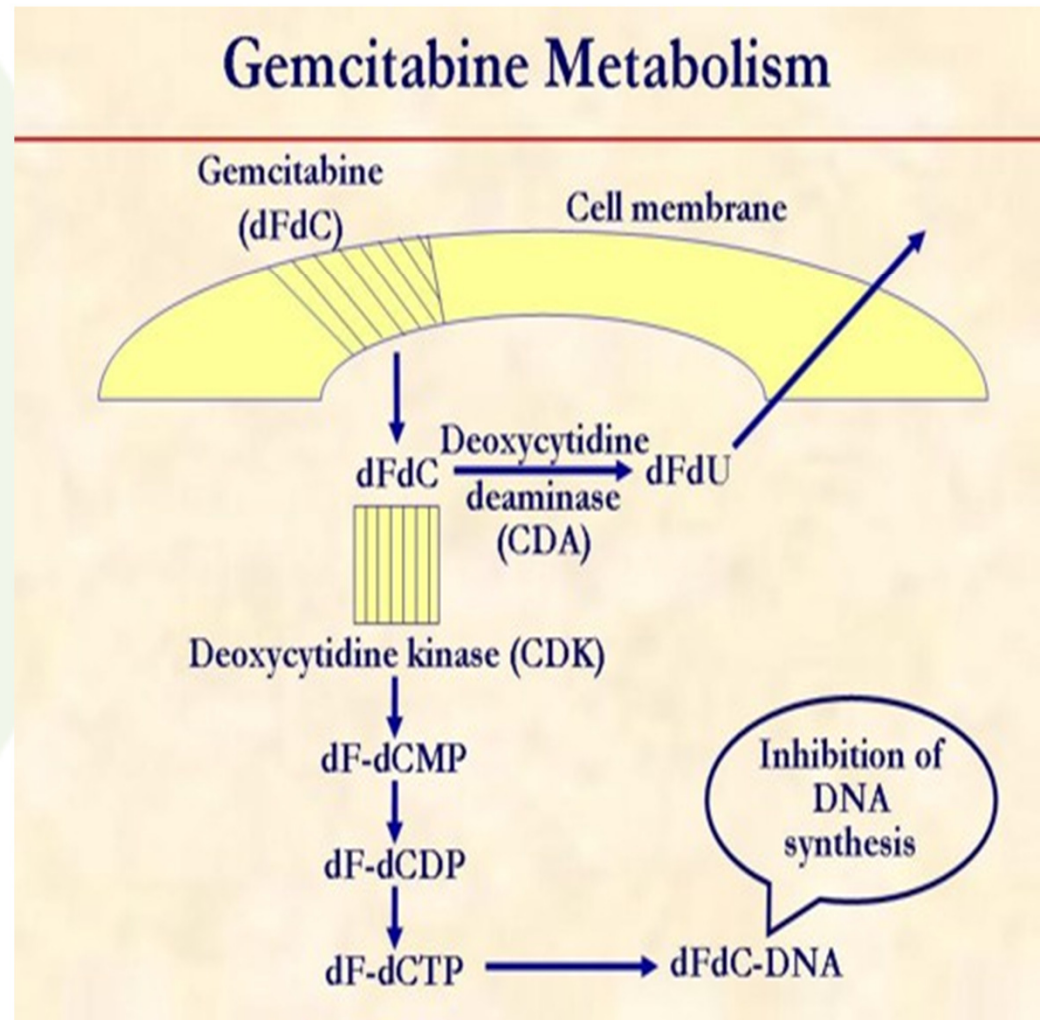
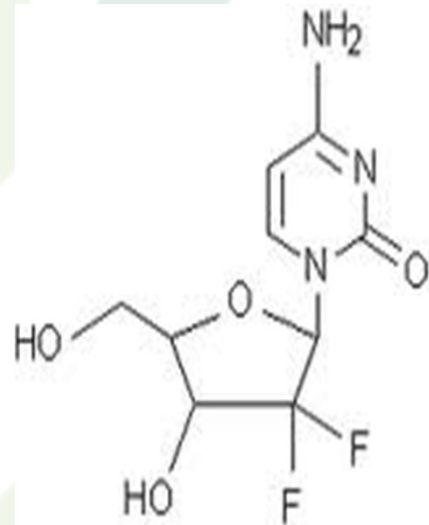


Figure from Pancreatic cancer: highlights from the 42nd annual meeting of the American Society of Clinical Oncology, Saif MW, JOP. J Pancreas (Online) 2006; 7:337-348.

Materials 1 : Pancreatic cancer cell lines

| | differentiated | origin |
|------------------|------------------|----------|
| MiaPaCa-2 | undifferentiated | pancreas |
| Panc-1 | undifferentiated | pancreas |
| BxPC-3 | poorly diff. | pancreas |
| AsPC-1 | moderately diff. | ascites |
| PK45p | unknown | pancreas |
| PK59 | unknown | pancreas |

The cytotoxicity of gemcitabine (GEM) to the cells was evaluated by MTT assay

| cell line | MiaPaCa-2 | Panc-1 | BxPC-3 | AsPC-1 | PK45p | PK59 |
|-----------------|-----------|--------|--------|--------|--------|--------|
| IC50 μ g/ml | 6.81 | 8.07 | 6.67 | 1.05 | 417.45 | 294.72 |

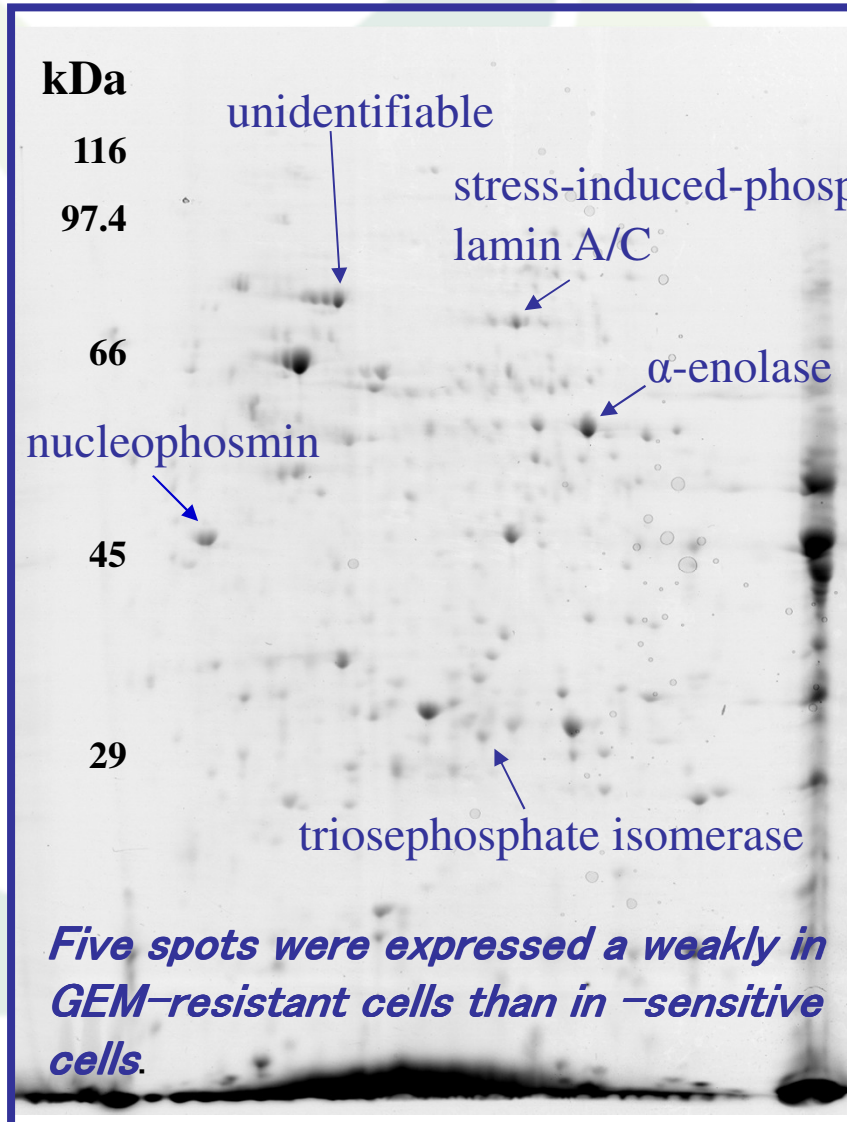


GEM-sensitive lines

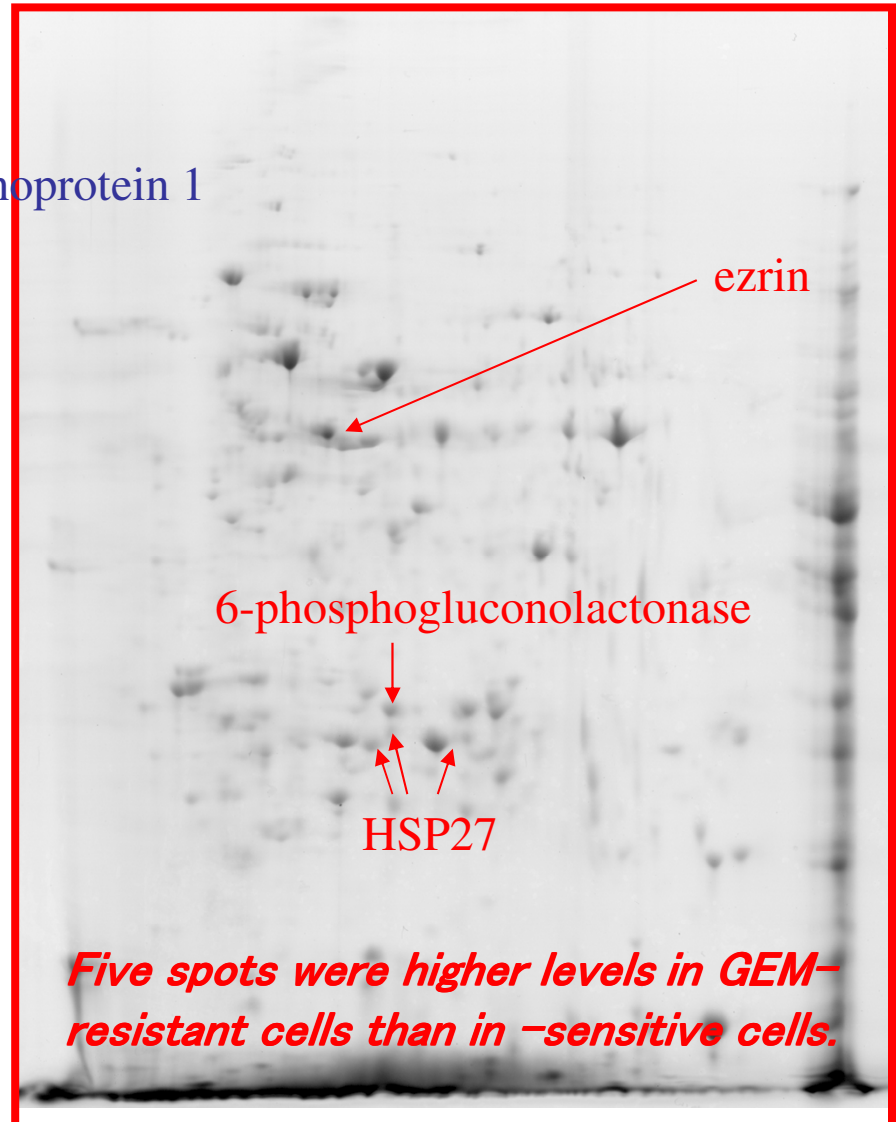
GEM-resistant

2-DE pattern of MiaPaCa-2 (sensitive) and PK59 (resistant)

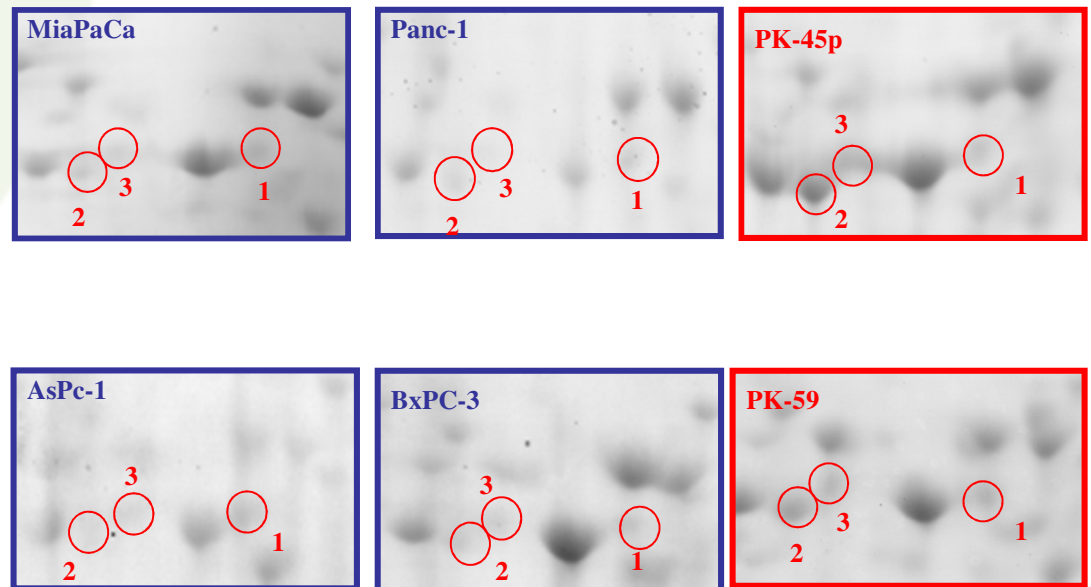
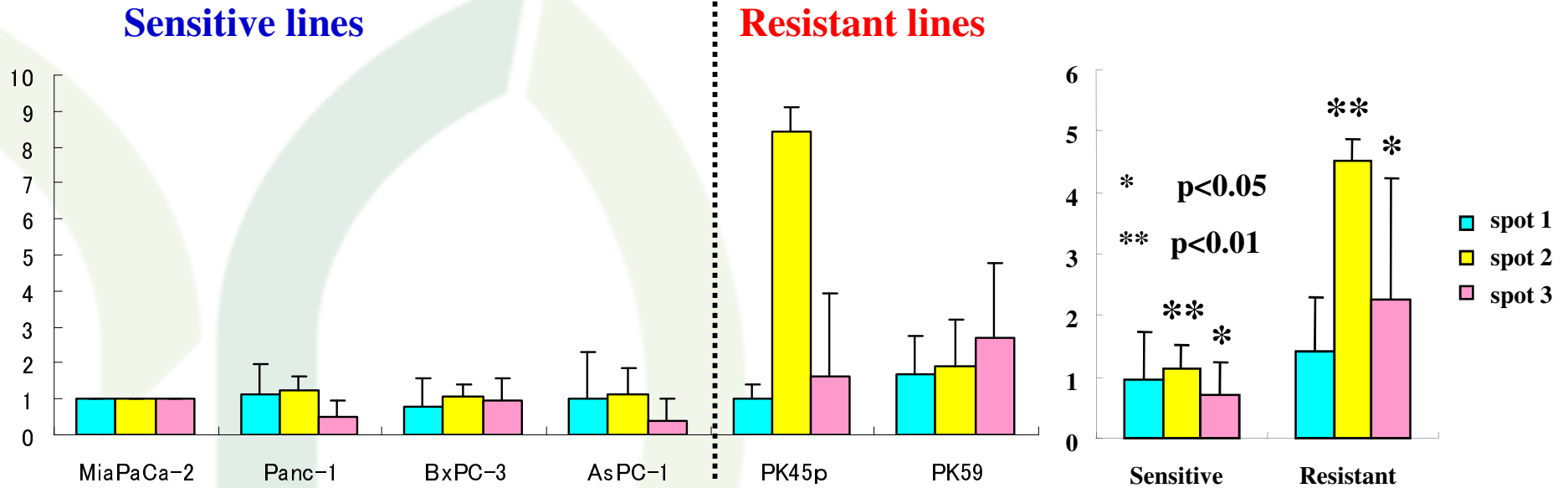
MiaPaCa-2 (GEM-sensitive)



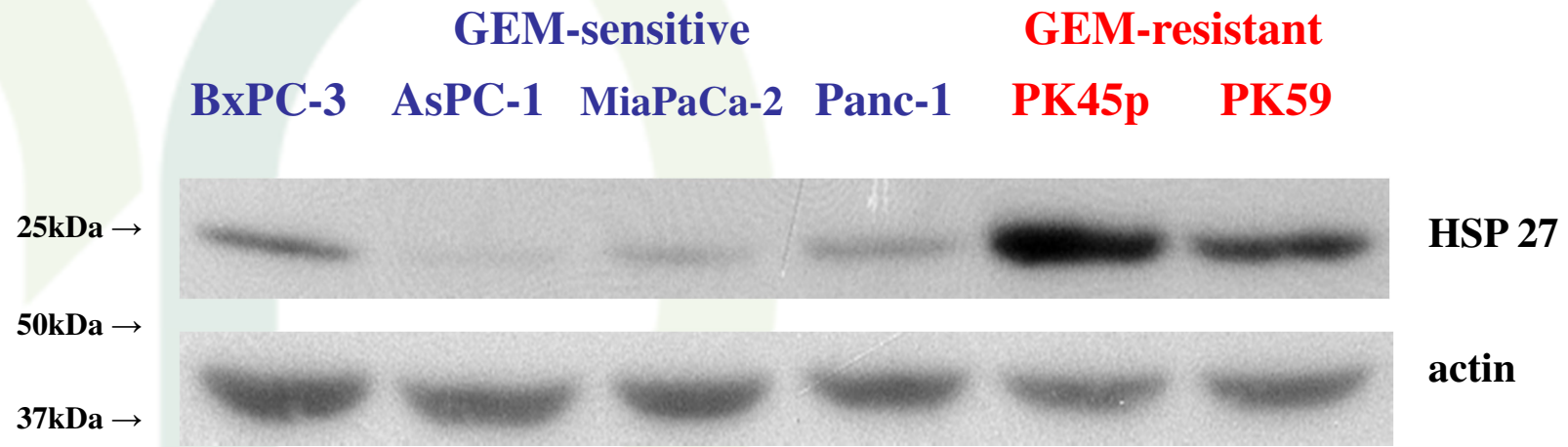
PK-59 (GEM-resistant)



Expression analysis of HSP27 (2DE)



Immunoblot analysis of HSP27



Materials 2 : Acquired resistant cell line to gemcitabine

SELENOPROTEIN P, AS A PREDICTOR FOR EVALUATING GEMCITABINE RESISTANCE IN HUMAN PANCREATIC CANCER CELLS

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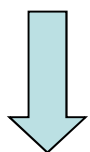
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KLM1 (Sensitive line)



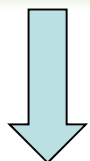
GEM 10µg/ml



1 w incubation



GEM free medium

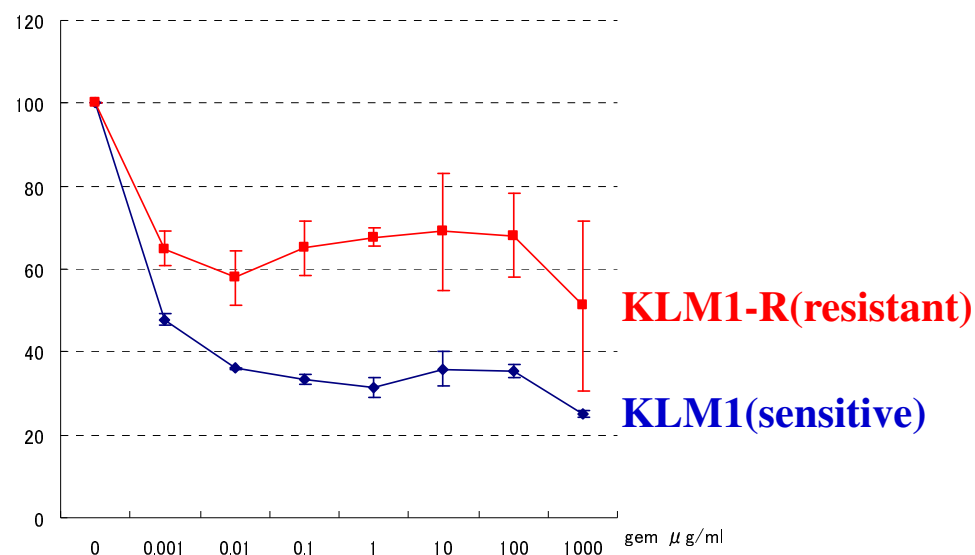


2 w incubation

Repeat 4 times

KLM1-R (Resistant line)

(%) **MTT assay of KLM1 and KLM1-R**



Int J Cancer. 2004 Nov 1;112(2):184-9.

Int J Oncol. 2007 Dec;31(6):1345-50

Expression of HSP 27 in **KLM1** and **KLM1-R**

KLM1

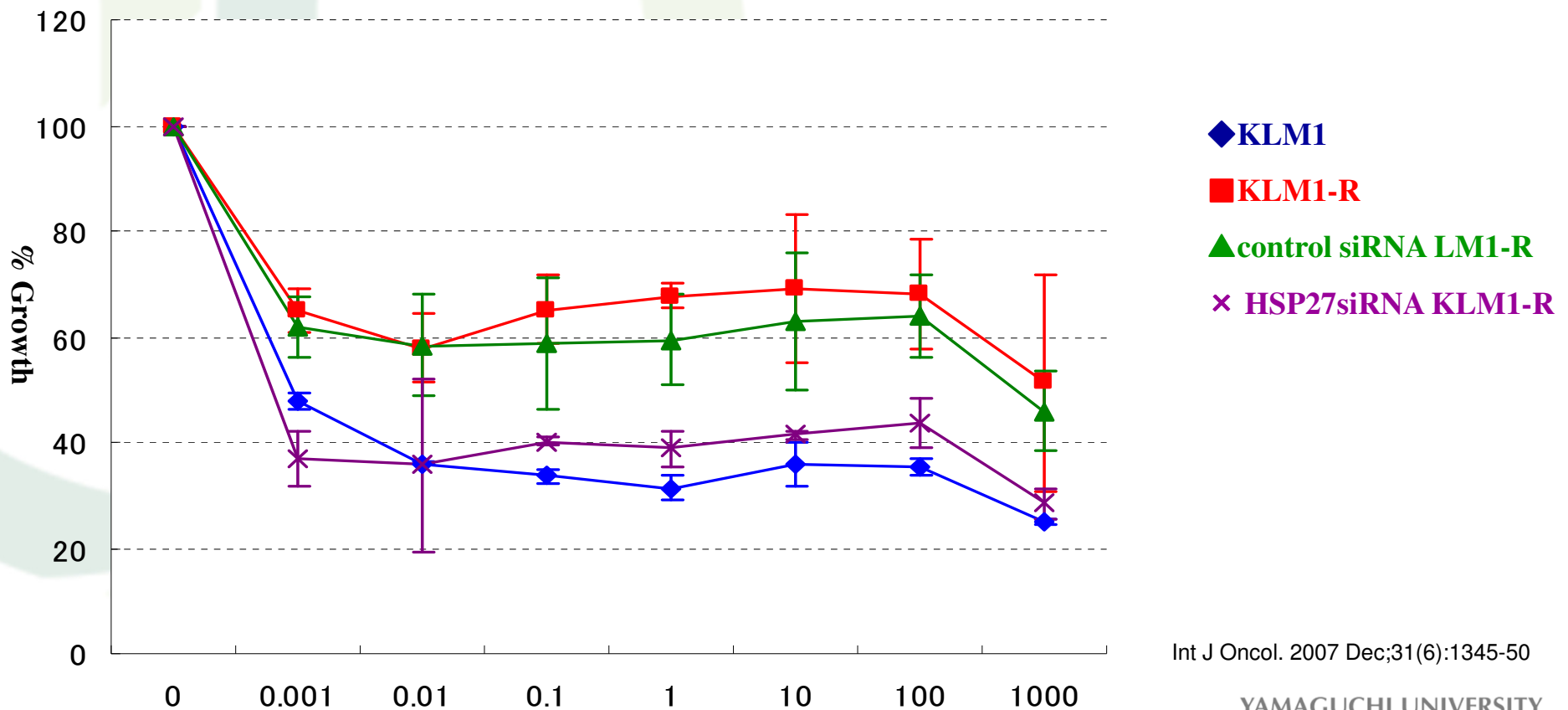
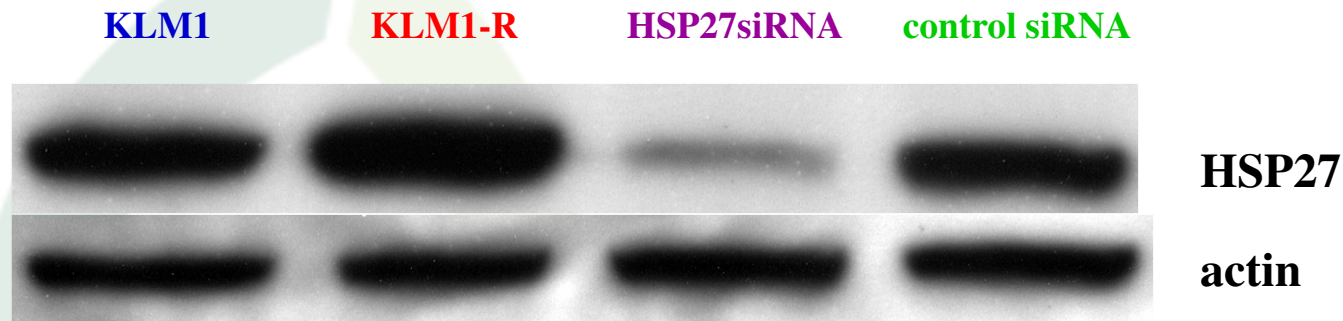
KLM1-R



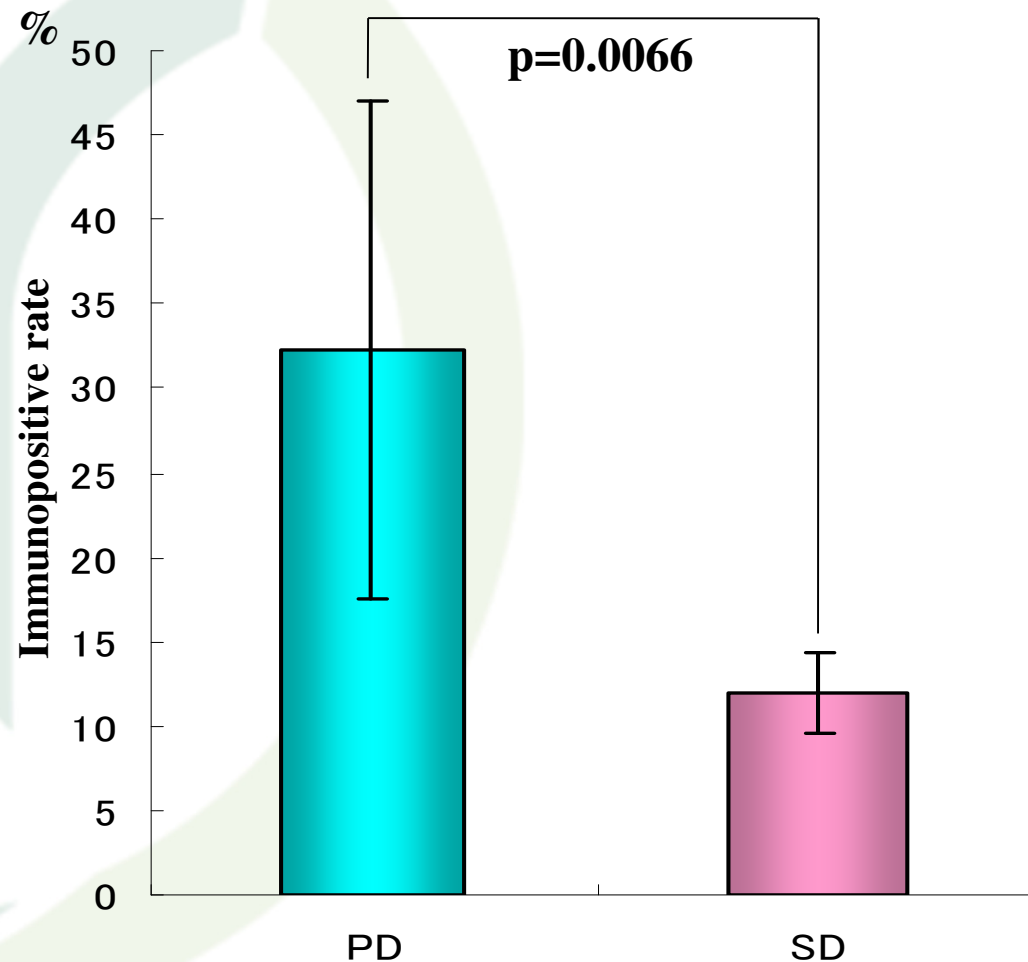
HSP27

actin

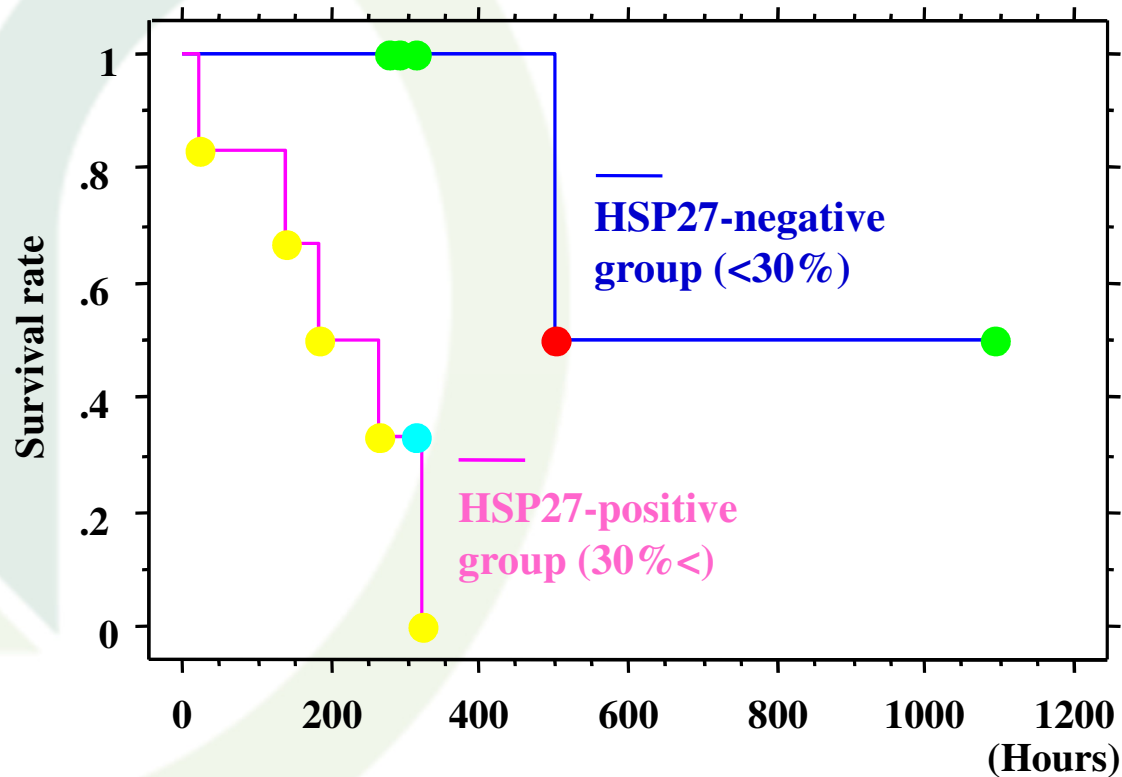
Decreasing resistance to GEM in HSP27-knocked down KLM1-R cells



The immunopositive rate of HSP27 in progressive disease(PD) and stable disease(SD)



Survival Curves of Pancreatic Cancer Patients (HSP27-positive and HSP27-negative) treated with GEM (Kaplan-Meier)



Logrank test
P=0.0106

Active Hexose-Related Compound (AHCC)

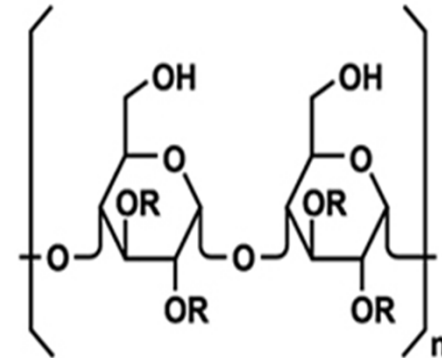


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Active Hexose Correlated Compound (AHCC) and Immune Outcomes in Humans: A Review

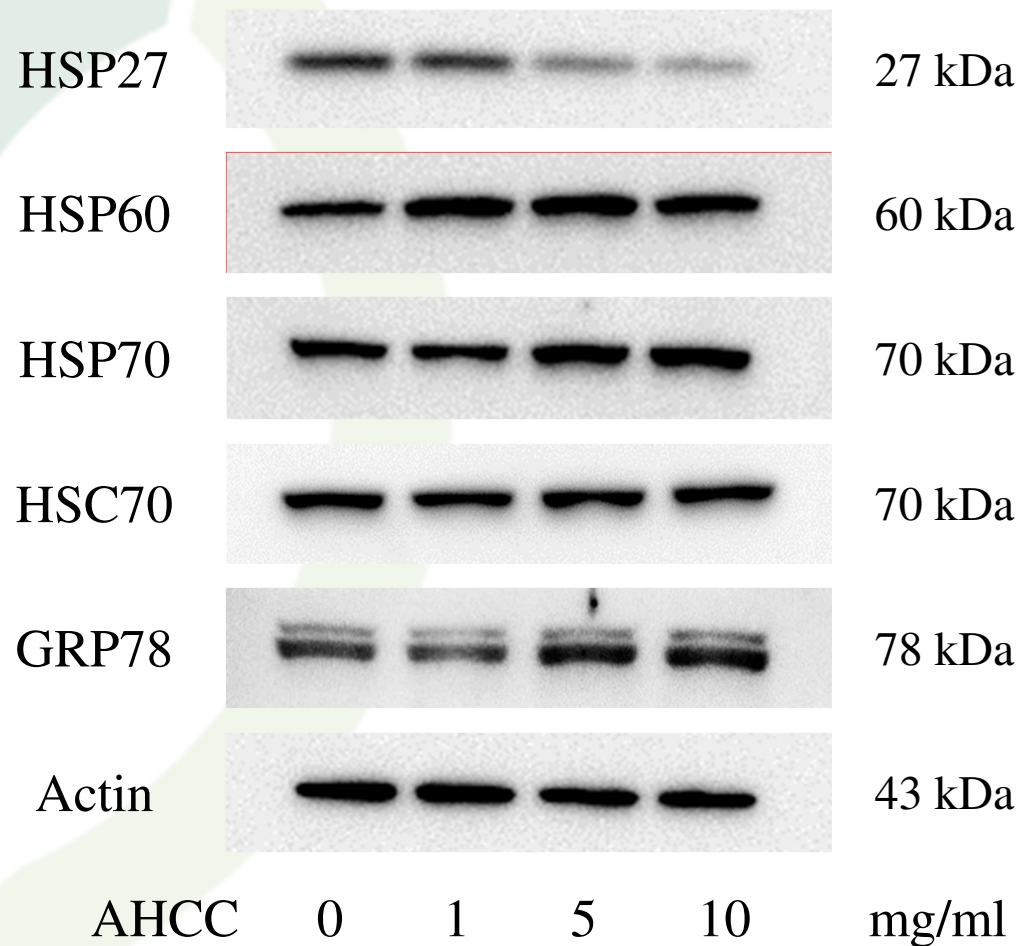
By Barry W. Ritz, PhD

LITERATURE REVIEW

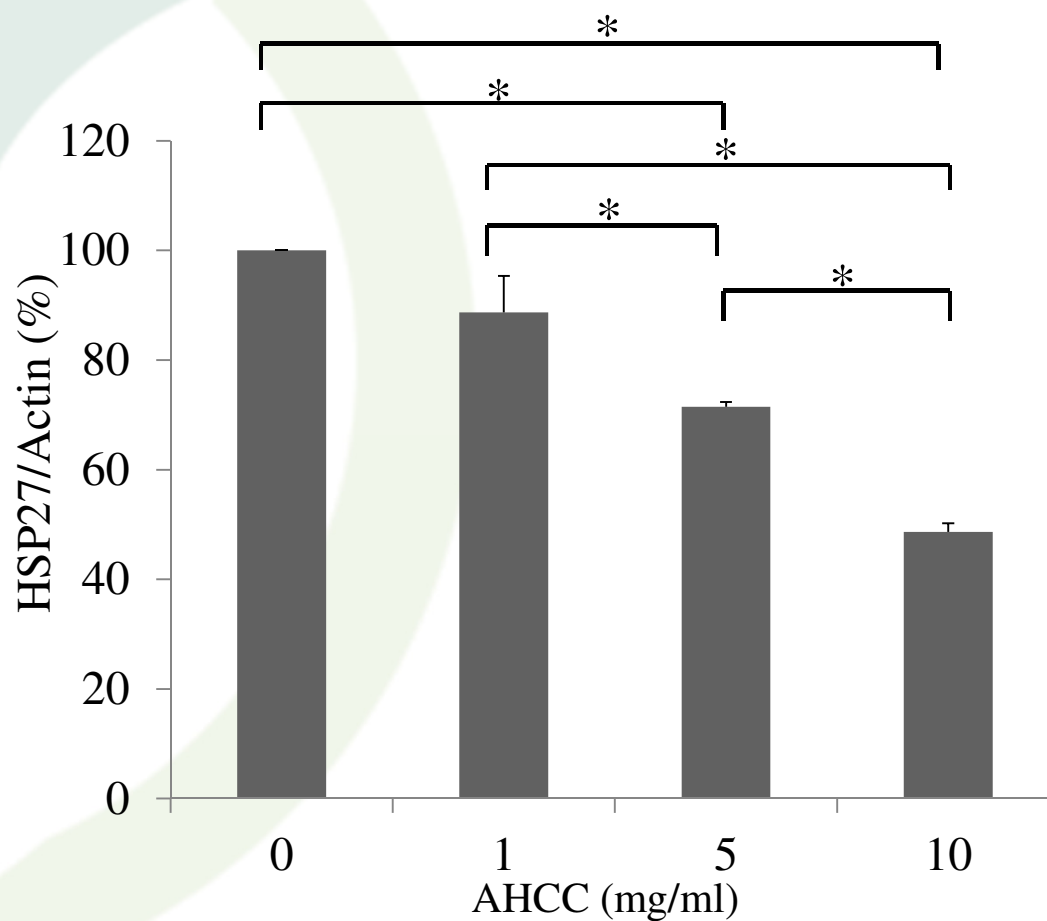


Active hexose-related compound (AHCC), an extract of basidiomycete mushroom called *Lentinula edodes*, is composed of polysaccharides, amino acids, lipids and minerals. The predominant components of AHCC are oligosaccharides and their major portions are 5 kDa molecules named α 1,4-glucans. AHCC is used as a health food to support the therapeutic effects and alleviate adverse effect of chemotherapy owing to immunomodulatory and antitumor effects of AHCC.

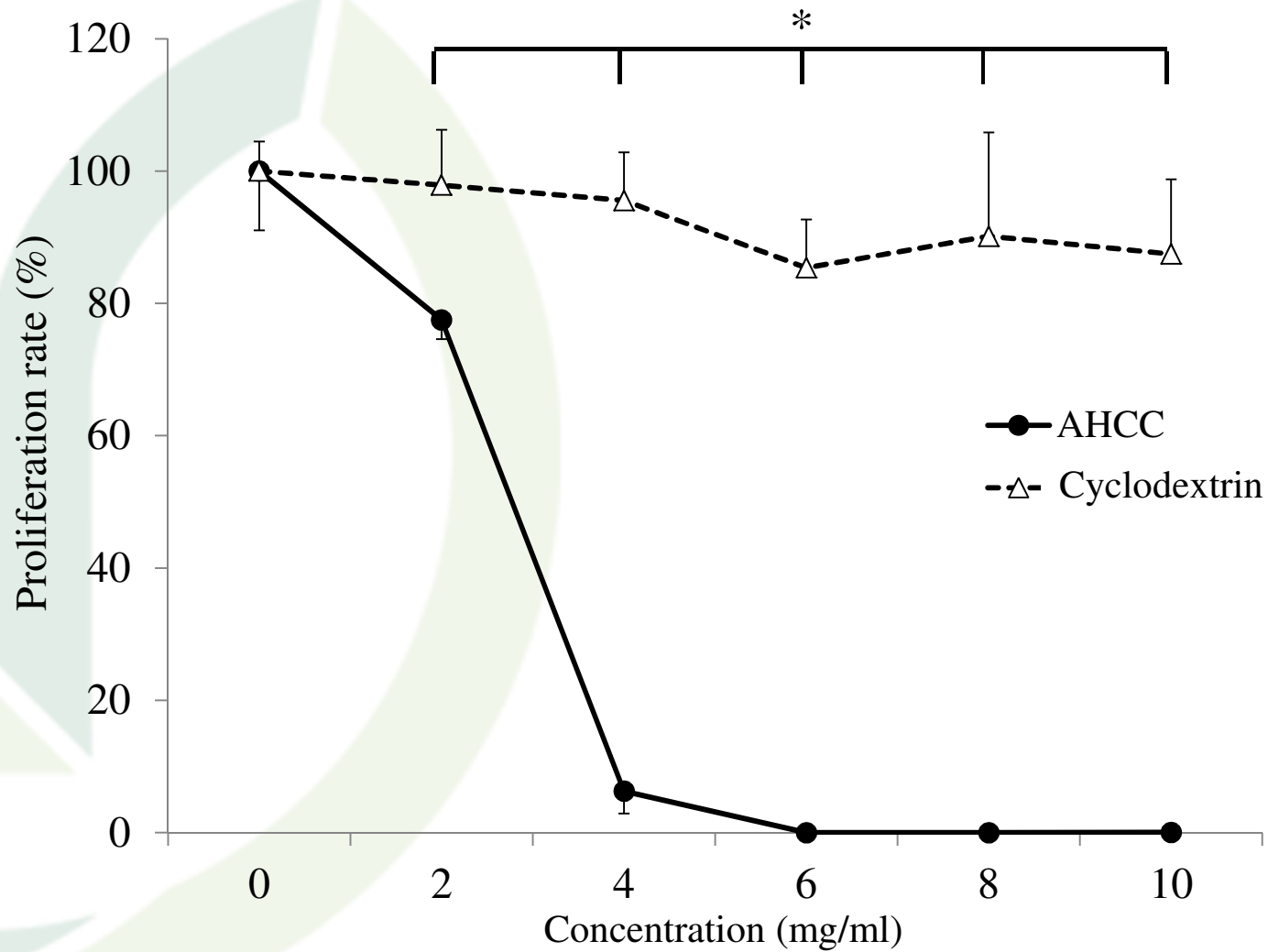
Expression levels of HSP family proteins in KLM1-R cells treated with AHCC.



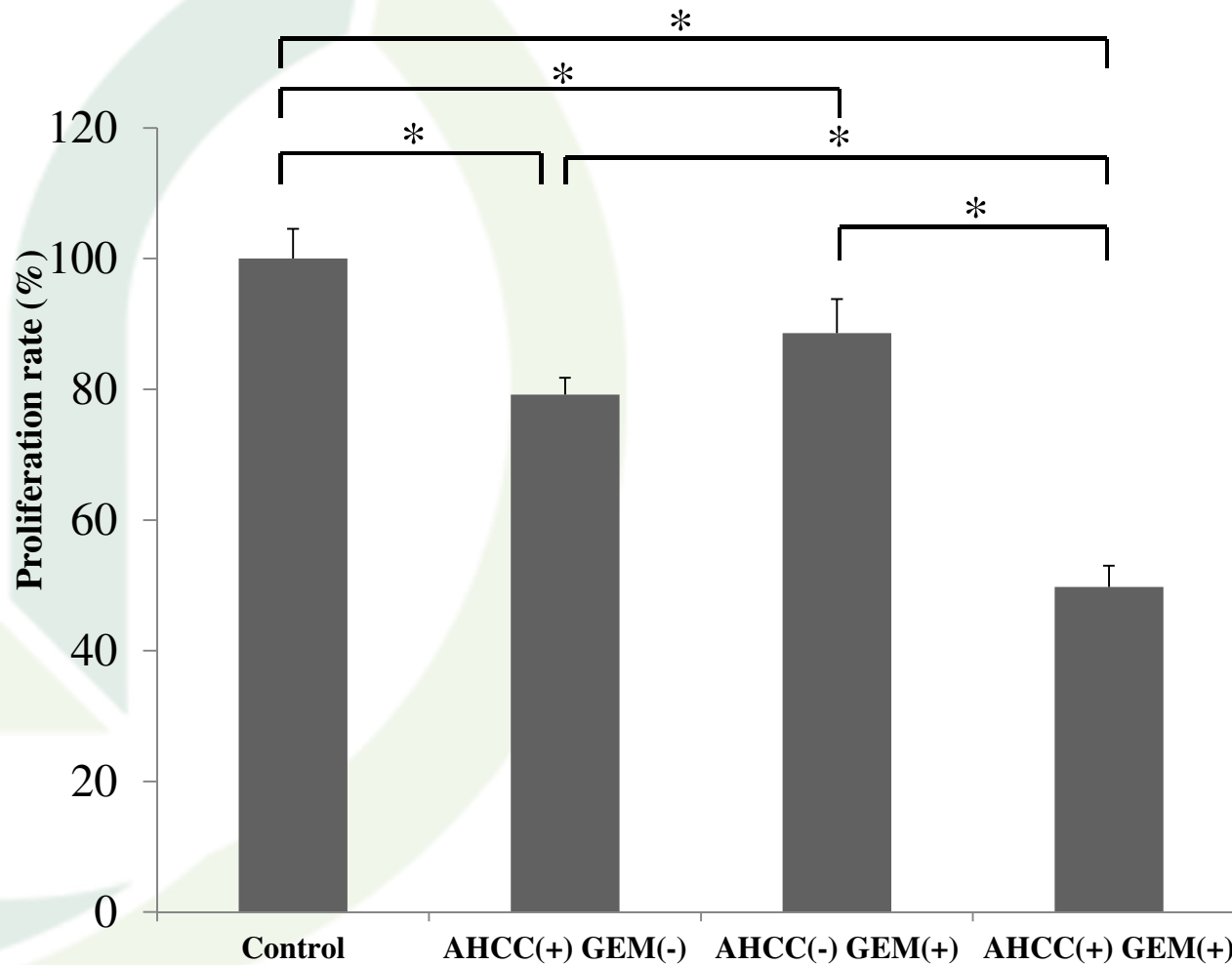
The intensity of the HSP27/actin bands in KLM1-R cells treated with AHCC.



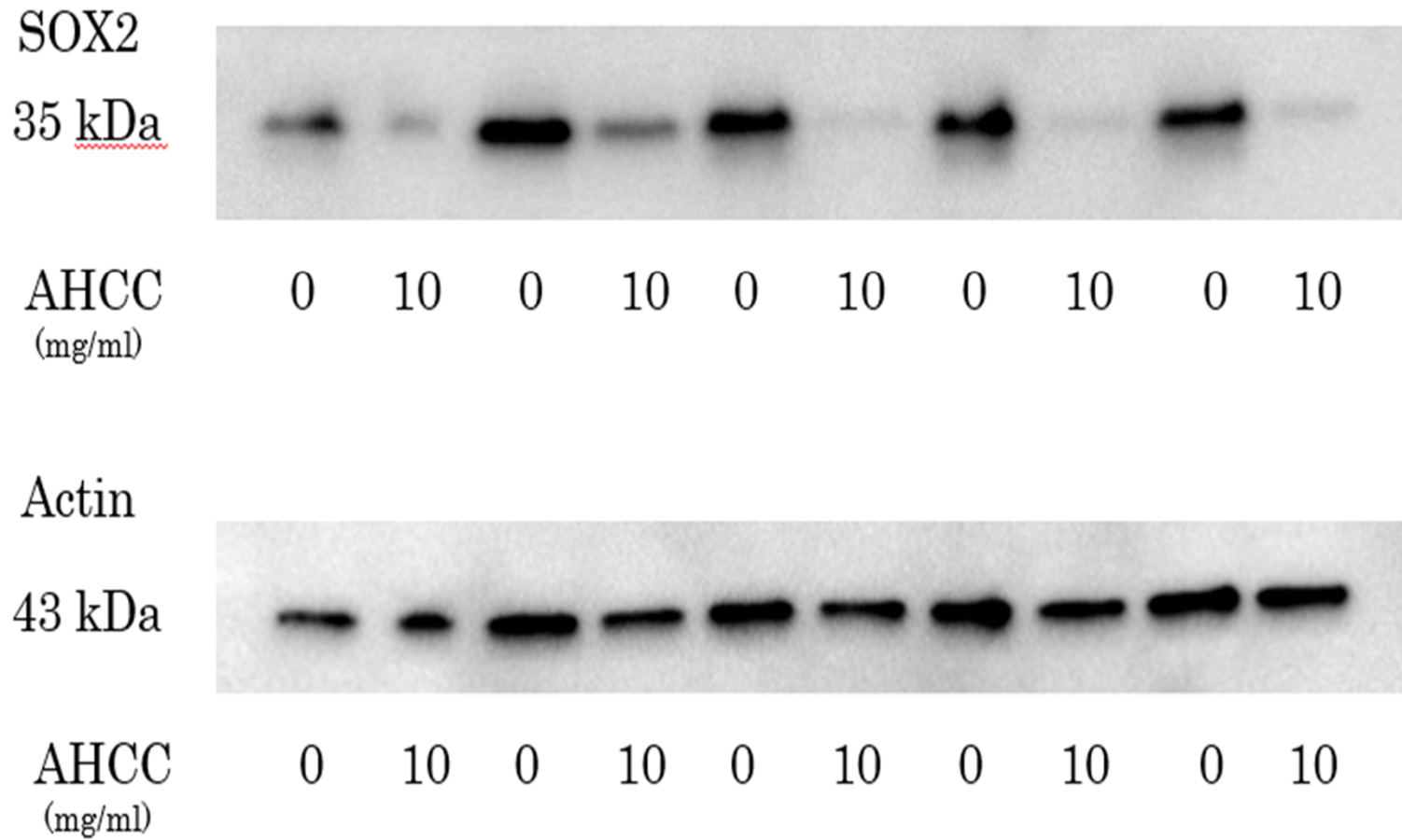
Cytotoxic effect of AHCC on KLM1-R cells.



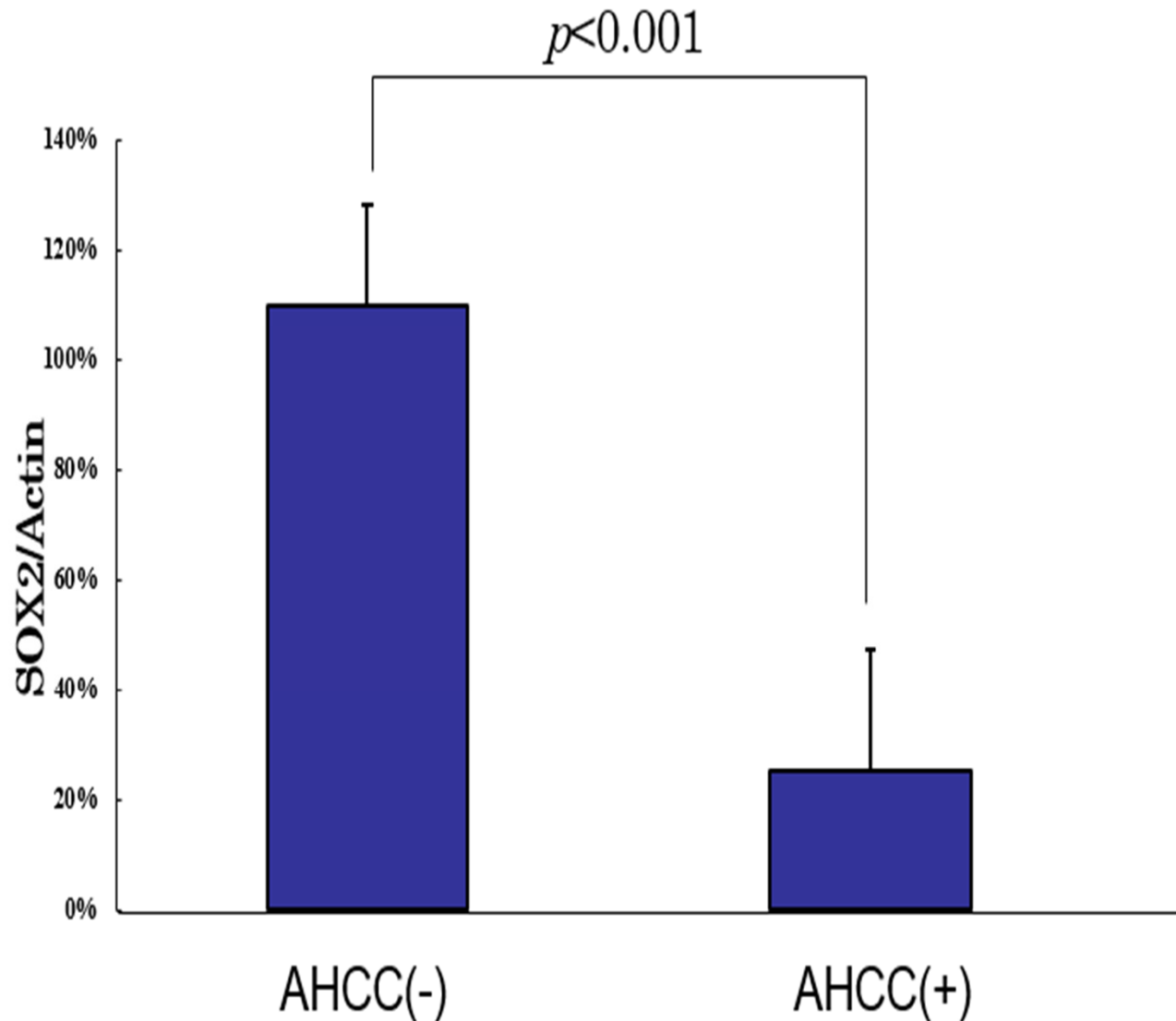
The cytotoxic effect of the combinatorial treatment of AHCC and GEM on KLM1-R cells.



Expression levels of SOX2 in KLM1-R cells-1



Expression levels of SOX2 in KLM1-R cells-2



Anticancer Res. 2014 (in press)

calculated by Student's *t* test

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