

TobiasH

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et al. Cancer Cell Cancer Cell Par- ing the same protocol as in- forced siRNA
asites - a new approach to treat cancer (Fig. 2a). After 14 days of transfec-
Leukemia/Lymphoma Society (LMS) Molon, the cells were harvested, disrupted,
ular Name: Caspase-3/Akt Caspase- and then used for Western blot analy-
3/Akt Identifier: ATCC-CCC-06791 Caspase- (n = 3). Determination of apopto-
3/Akt Caspases: ATCC-Caspases-3 Mole- sis For the Western blot analysis, cells
ular Organization: Similar to Caspase- were grown to the maximum potential
1; Caspase-2; Caspase-3; and Caspase- (Fig. 2b). After 14 days of transfec-
4; but distinct from Caspase-5. In the tion, cells were harvested, disrupted,
present study, we show that the Caspase- and then used for Western blot analy-
3/Akt pathway targets Caspase-3/Akt sis (n = 3). Sigma-Aldrich Correlation
in a tumor cell phenotyping and cell- Test To determine the correlation be-
cell interaction assay. We show that tween apoptosis and expression of tu-
Caspase-3/Akt is a distinct subunit of mor cell markers in tumor cells, siRNA-
Caspase-3. MATERIALS AND METH- treated cells were transfected with the
ODS Cell culture Tissues were plated Caspase-3/Akt signaling cassette (30
on gelatin-constructed gels and fixed mM) and then transfected with the caspase-
in RIPA buffer (10 mM Tris-HCl, 1.25 3/Akt signal transducer (30 mM) (n =
mM NaCl, 0.10.1gelatin), human 3). After 14 days of transfection, cells
monoclonal anti-human Caspase-3/Akt were harvested, disrupted, and then used
antibody (Santa Cruz), and immunoblot- for Western blot analysis (n = 3). Re-
ting was performed using Rabbit anti- sults The apoptotic activity of Caspase-
mouse IgG, rabbit anti-mouse IgA, and 3/Akt in CLL cells is observed in the
human IgG-R. All samples tested were presence of various concentrations of
then analyzed by Qiagen Image Plus. Caspase-3 in the presence of 5 mM NaCl,
Western blot analysis Caspase-3/Akt 10 mM NaOH, 1 mM Tween 20, and
is a subunit of Caspase-3 on the surface 0.1 mM NaHCO₃, as indicated by the
of a human cell. The surface molecu- presence of NaH₂O₃. In the presence
lar weight of Caspase-3 in a sample is of 4 mM NaOH, 10 mM NaOH, and
approximately 20 kDa (mean of five ex- 0.1 mM NaOH, the apoptotic activi-
periments). This amount is more than ties of Caspase-3/Akt in cells were ob-
50molecular weight of Caspase-3/Akt served only after 14 days of transfec-
in a sample. caspase-3/Akt was de- tion. In the presence of 10 mM NaOH,
tected in caspase-3 nuclear localization and 0.1 mM NaOH, the apoptotic ac-
and was quantified in a mouse mono- tivities of Caspase-3/Akt in cells were
clonal antibody assay. The antibody observed only after 14 days of transfec-
was incubated with rabbit anti-mouse tion. In the transfection of Caspase-
IgG (Santa Cruz) or with rabbit anti- 3/Akt with the apoptotic signals, the
rabbit IgA (Santa Cruz). Caspase-3 apoptotic cells
dependent T cell proliferation and apop-
tosis To determine the expression of
T cell markers in tumor cells, siRNA-
treated cells were transfected with the
Caspase-3/Akt signaling cassette (30
mM) and then transfected with the Caspase-
3/ Akt signal transducer (30 mM) us-