Hücre Ölümünün Tıp ve Biyolojik Bilimlerdeki Önemi ve İlgili Serum Belirteçleri

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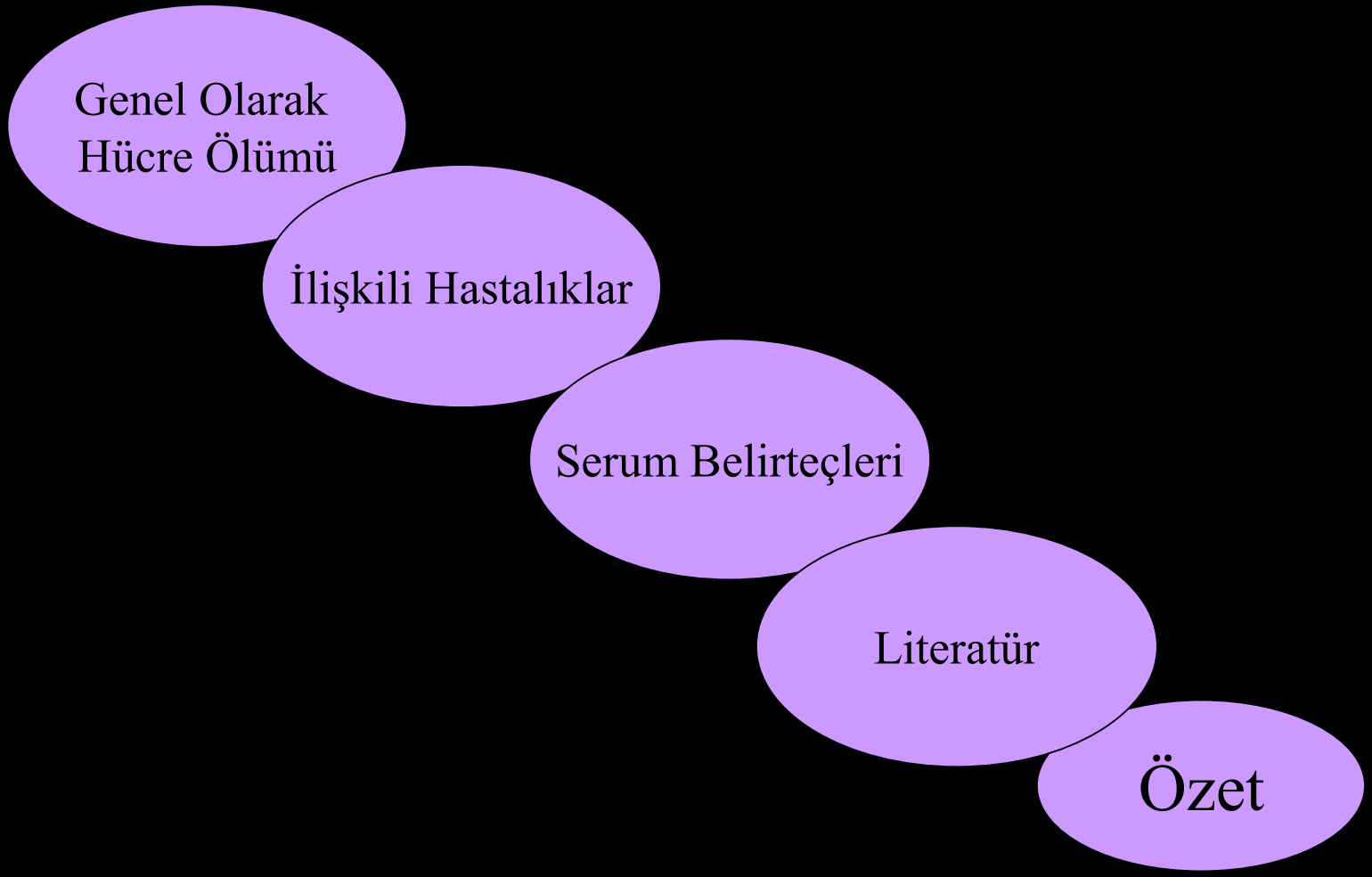
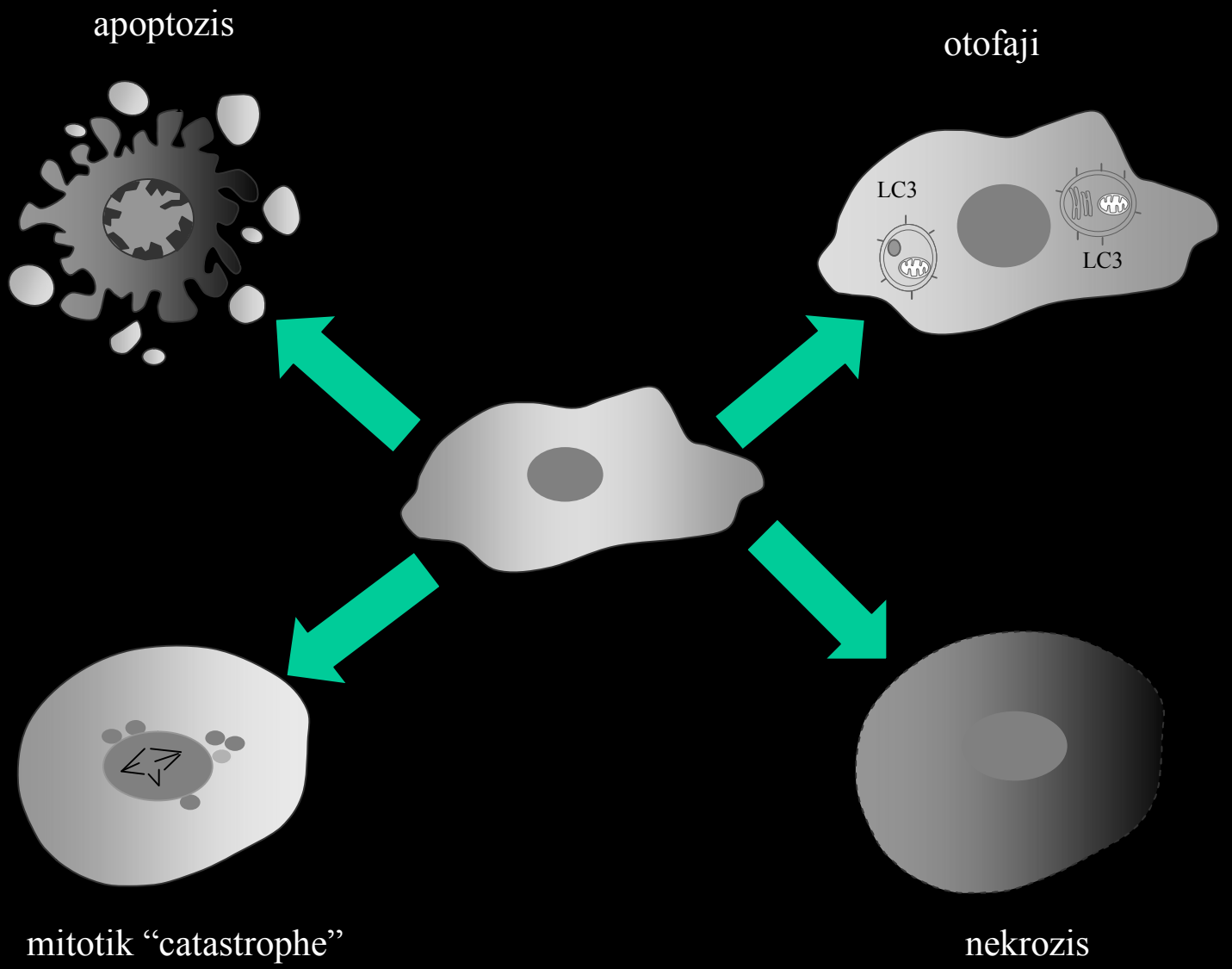
BURSA

Konuşmanın İçeriği



Hücre Ölüm Şekilleri



Opinion

Trends in Cell Biology July 2011, Vol. 21, No. 7

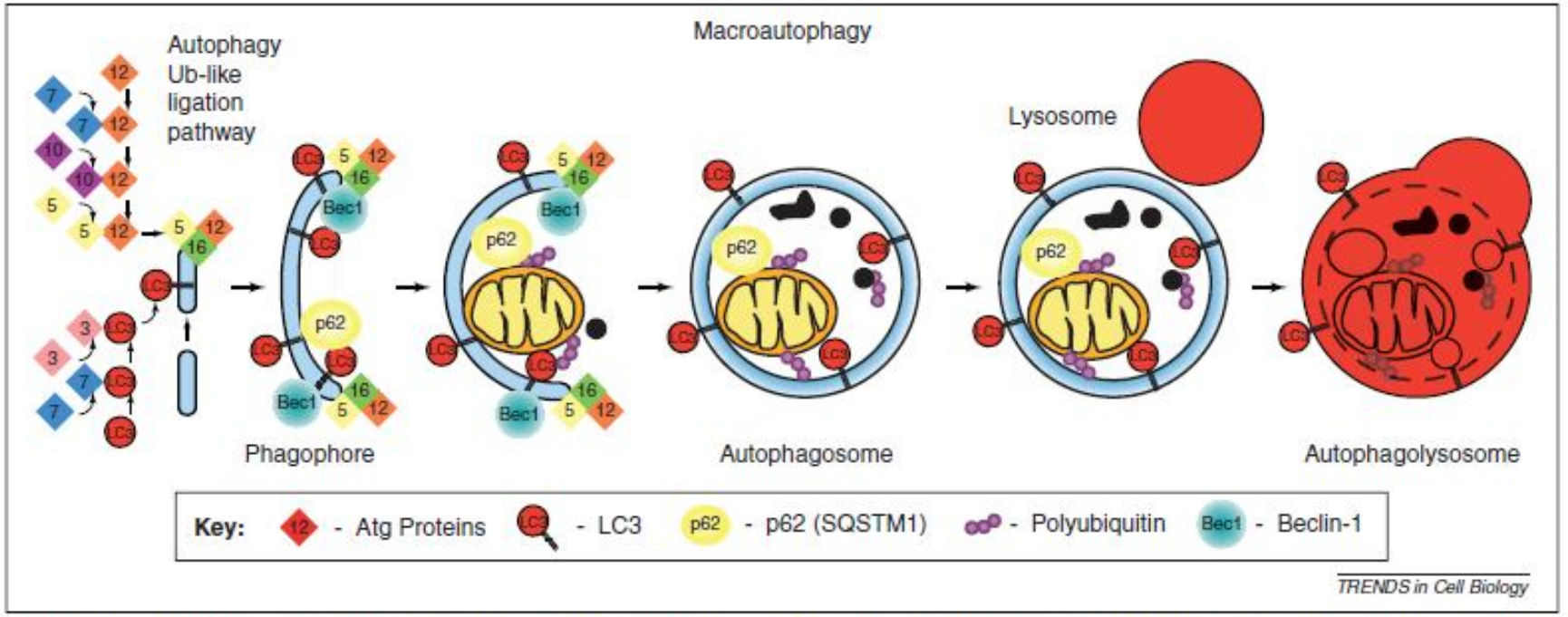
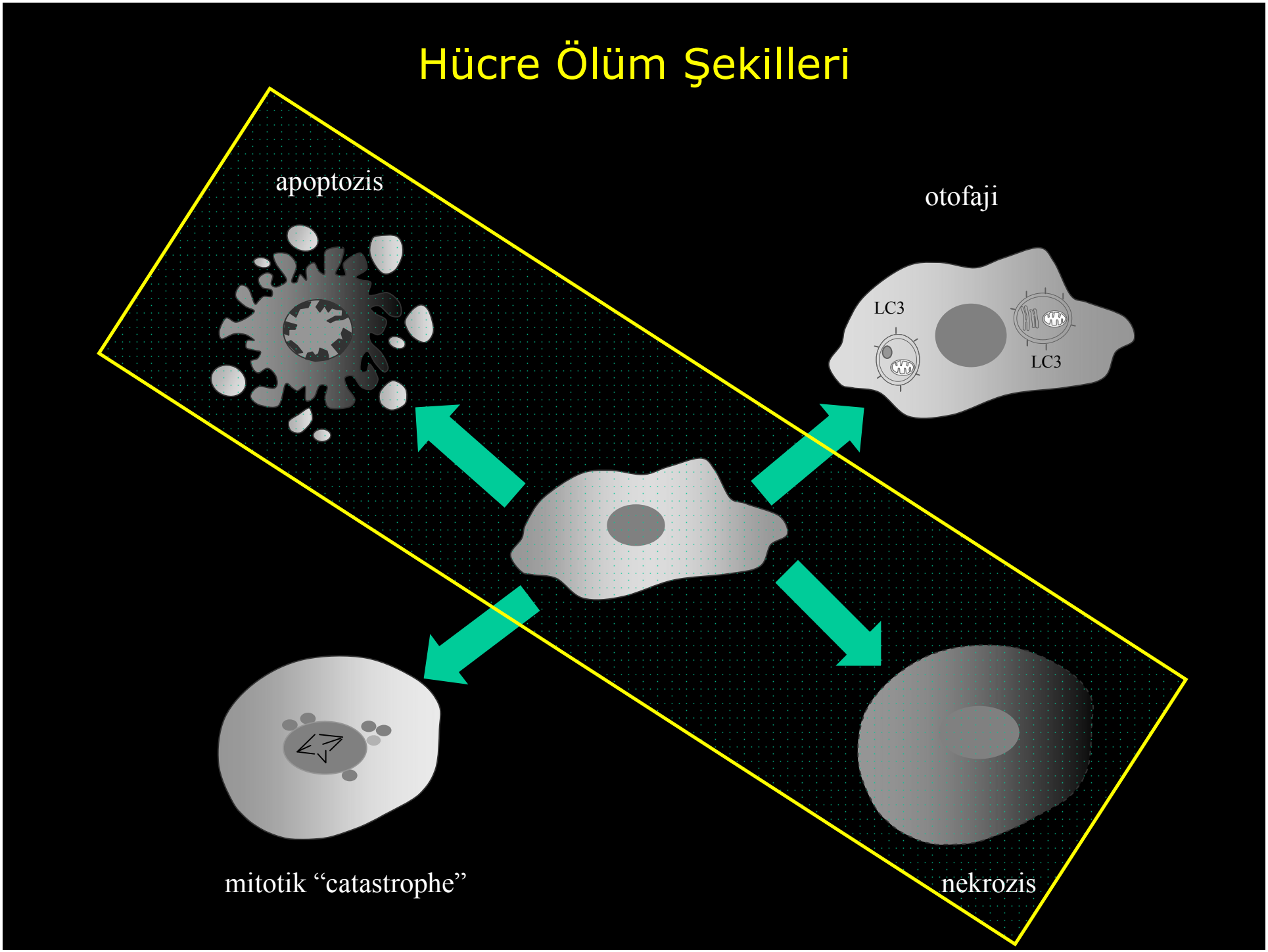
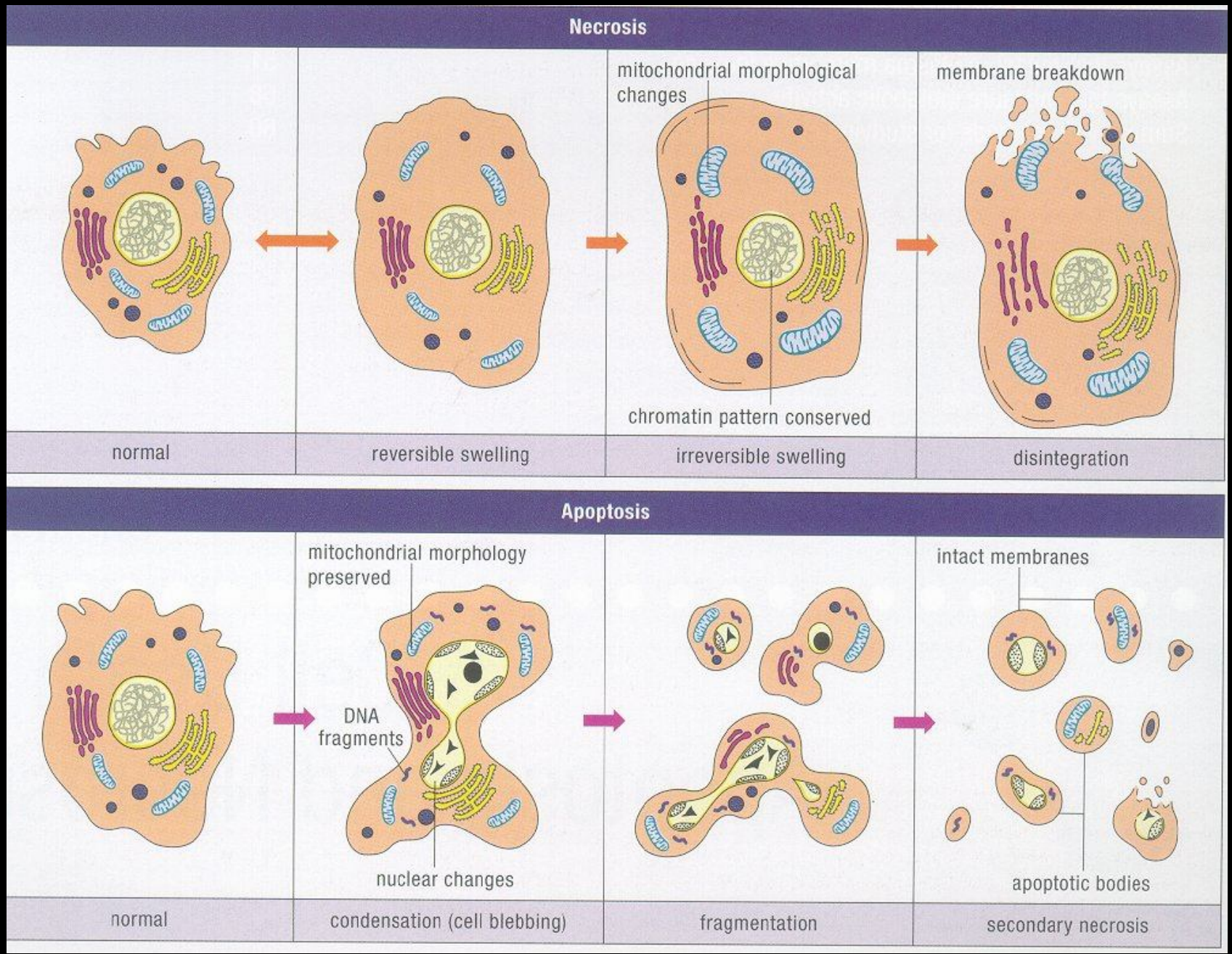


Figure 1. Macroautophagy. The autophagic process degrades cellular macromolecules and organelles, releasing metabolites to provide the cell with energy and anabolic building blocks to aid in survival and repair during nutrient deprivation or cellular damage. The Ubiquitin-like conjugation cascade leads to the lipidation of LC3 and conjugation of Atg5-Atg12, two key elements in the formation of the phagophore. The autophagic process begins (autophagy induction) with formation of the phagophore, followed by its elongation and closure to form the autophagosome that then fuses with lysosomes, resulting in degradation of the contents (autophagic flux).

Kanser, Nörodejenerasyon, Yaşlanma, Otoimmun hast. (Crohn's disease, romatoid artrit), Kalp hast. İnfeksiyon


Hücre Ölüm Şekilleri
apoptozis
otofaji
LC3
LC3
mitotik “catastrophe”
nekrozis


Necrosis
mitochondrial morphological changes
membrane breakdown
chromatin pattern conserved
normal
reversible swelling
irreversible swelling
disintegration
Apoptosis
mitochondrial morphology preserved
intact membranes
DNA fragments
nuclear changes
apoptotic bodies
normal
condensation (cell blebbing)
fragmentation
secondary necrosis

I. Amelio et al. / Biochemical and Biophysical Research Communications 414 (2011) 277–281

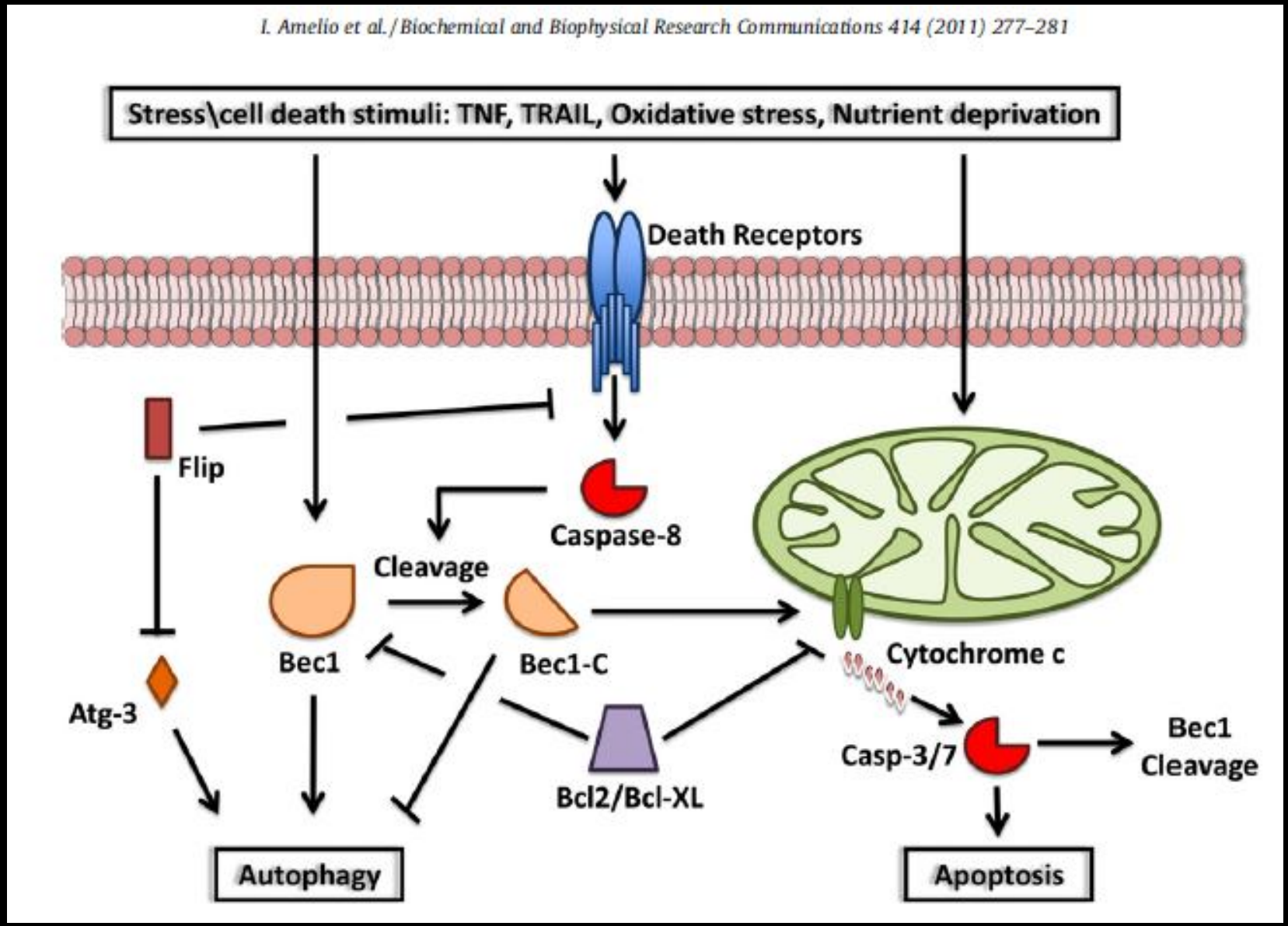


Table 1. Proteins with dual roles in autophagy and apoptosis

Protein	Function
DAPK	Phosphorylates Beclin1; activates DISC
RIP	Activates cell death independent of caspases; may activate autophagic cell death
NF-κB	Regulates survival pathways – inhibits apoptosis activates autophagy
JNK	Positively regulates both apoptosis and autophagy
p62	Crucial for activation of Caspase-8; regulates selective autophagy of many substrates
Beclin1	Primary cellular activator of autophagy; regulated by Bcl-2
Bcl-2	Inhibits both autophagy and apoptosis by binding Beclin-1 and Bax/Bad/Bak
Caspase-8	Activates apoptosis via extrinsic pathway; cleaves p62 during apoptosis
Caspase-3	May cleave Beclin-1 to inhibit autophagy during terminal stages of apoptosis
p53	Induces MOMP in response to stress; positively and negatively regulates autophagy
Atg5	Crucial autophagy gene; activates apoptosis via FADD and MOMP upon calpain cleavage
FLIP	c-FLIP inhibits autophagy through inhibition of Atg3-LC3 conjugation
Atg12-Atg3	Novel regulator of mitochondria and apoptosis with no known function in autophagy

Apoptozis nedir?

Sizler bu cümleyi okumayı bitirdiğinizde onbinlerce hücrenizi kaybetmiş olacaksınız.

Telaşlanmayın lütfen...☺

O hücreler sizin sağlıklı kalmanız için intihar ettiler.
"Cell suicide"

Fizyolojik şartlar altında öldüler.

Physiological cell death"

Üstelik, ölecekleri önceden programlanmıştı.
“Programmed cell death”

Cells are born, live for a given period of time and then die*

Bowen, 1998

APOPTOSIS

--- Cell suicide
--- Physiological cell death
--- Programmed cell death
--- Cell deletion

Her yerde APOPTOZİS !

Sağlıkta

... Embriyonun gelişimi
... Doku homeostazisi
... İmmünoloji

Hastalıkta

... Viral infeksiyonlar
... Nörodejeneratif hastalıklar
... Organ transplantasyonları
... İnsüline bağımlı tip diyabet
... AIDS
... Atherosklerozis
... Miyokard infarktüsü
... Otoimmün hastalıklar
... *Kanser oluşumu ve tedavisi*

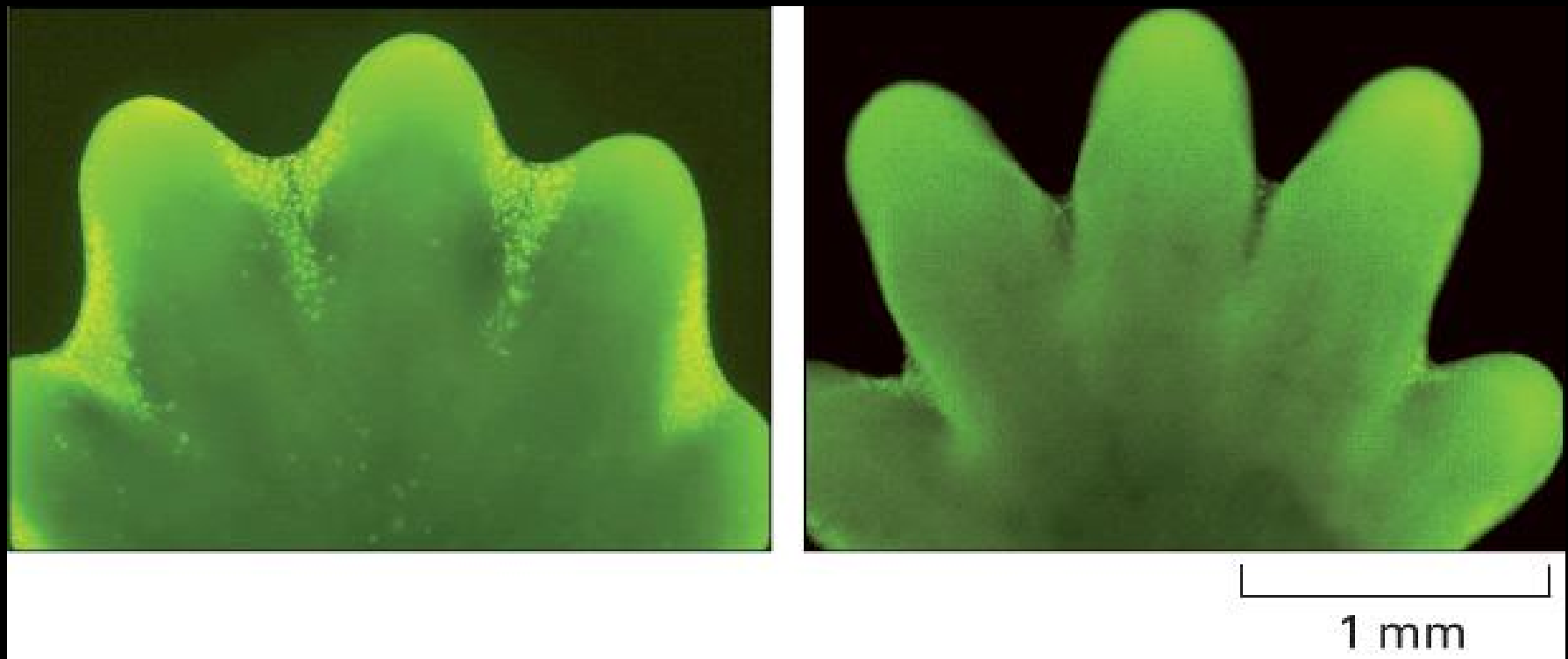
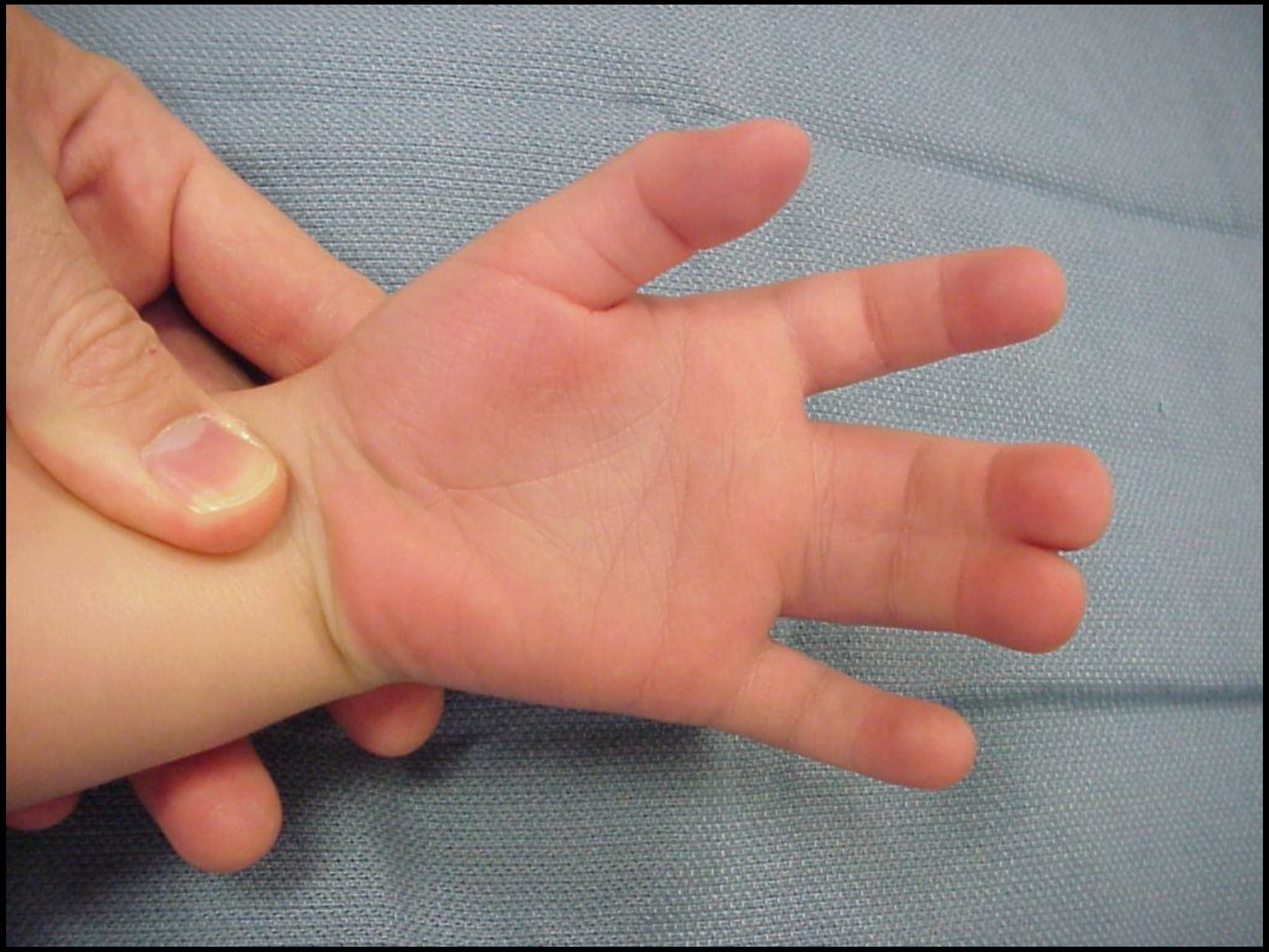
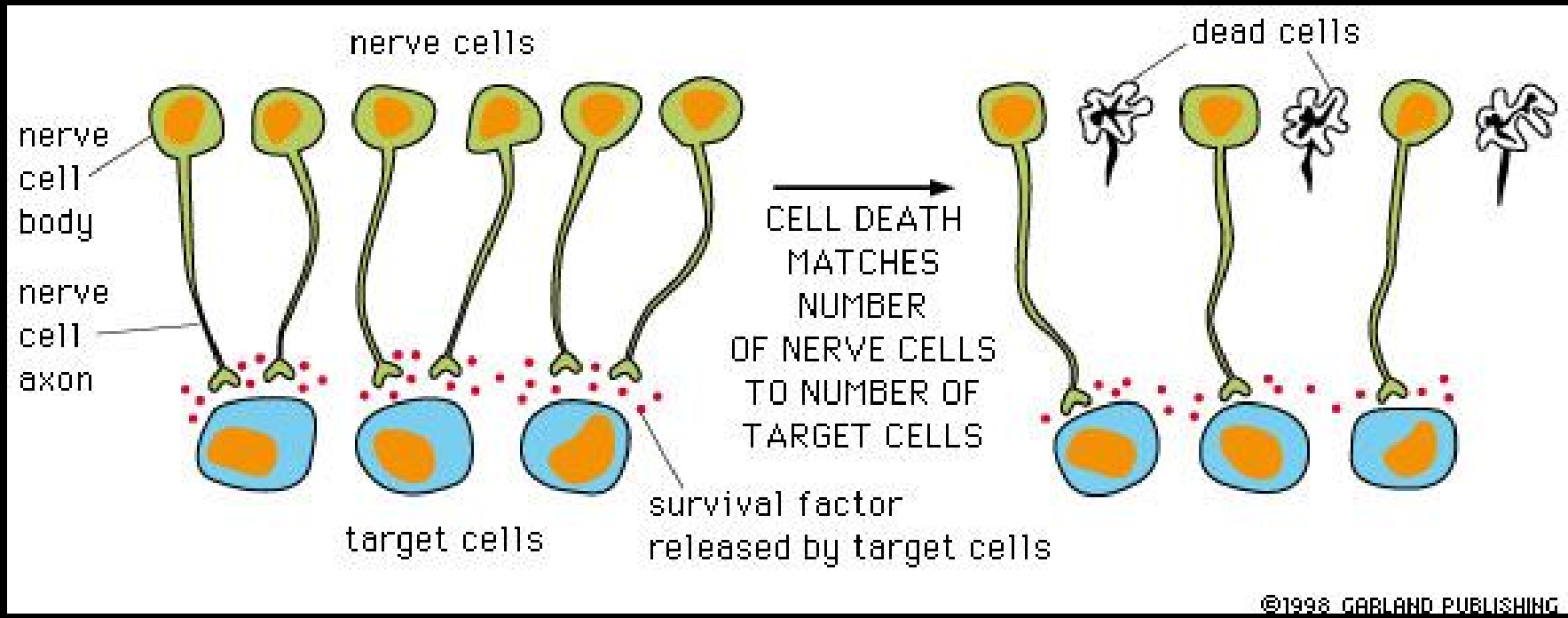
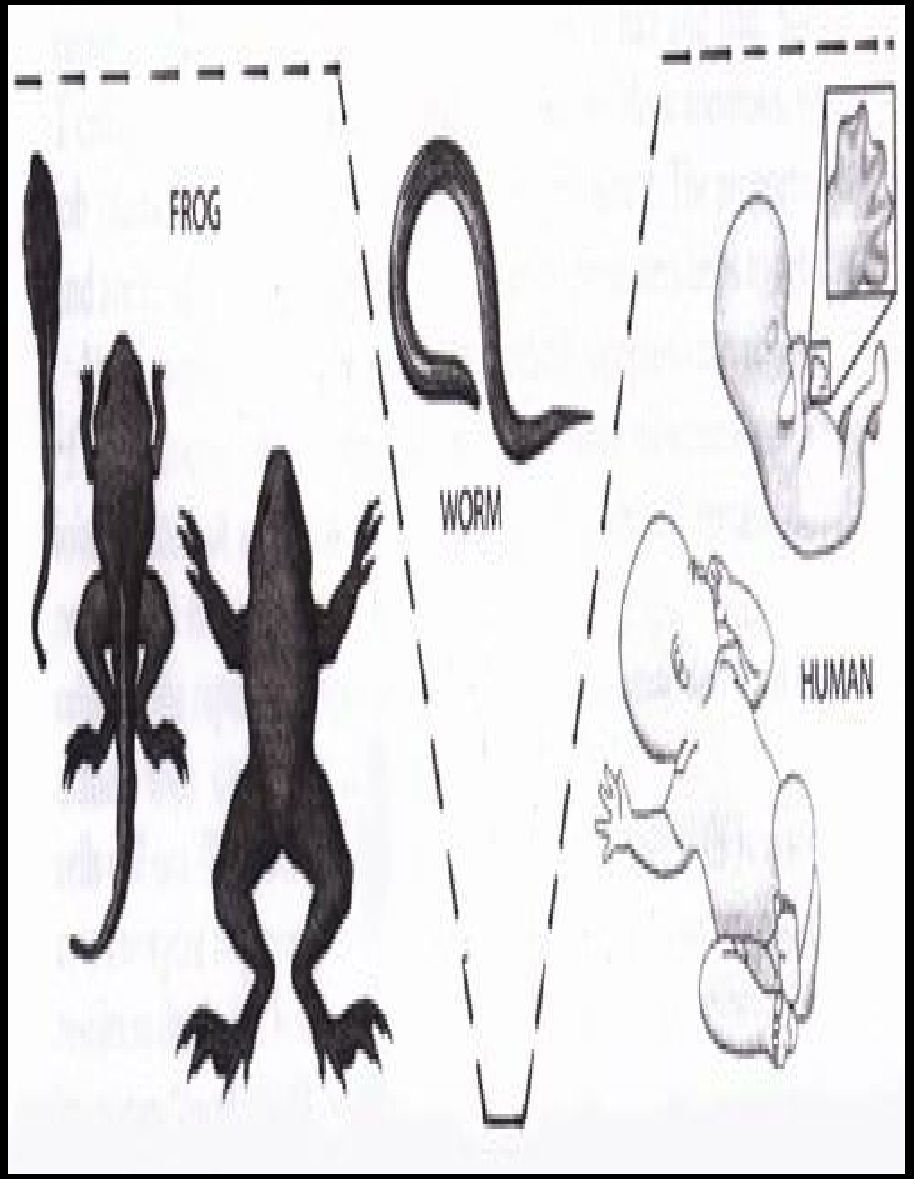


Figure 17–35. Molecular Biology of the Cell, 4th Edition.




nerve cells
dead cells
nerve cell body
nerve cell axon
CELL DEATH MATCHES NUMBER OF NERVE CELLS TO NUMBER OF TARGET CELLS
target cells
survival factor released by target cells
©1998 GARLAND PUBLISHING



YAKIN PLAN

Hürriyet

Nobel Tıp Ödülü kazandıran kurtçuk

Nobel Tıp Ödülü'nü, organ gelişiminde genlerin oynadığı rolle ilgili olarak 959 hücreli şeffaf kurtçuk üzerinde araştırmalar yapan ikisi İngiliz, biri Amerikalı üç bilimadamı kazandı. Bu araştırma çeşitli hastalıklara karşı yeni tedavi yöntemi geliştirilmesine olanak tanıyacak.

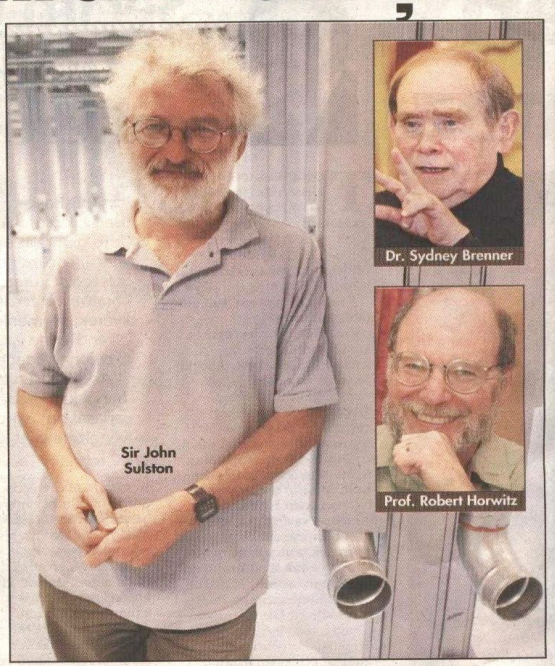
MINIK şeffaf bir kurtçuk üzerinde yaptıkları çalışmalarla genlerin, organların ve vücudun oluşumunu nasıl etkilediklerini ortaya çıkaran ikisi İngiliz, biri Amerikalı üç bilimadamı bu yılki Nobel tıp ödülüne layık görüldü.

"Kurtçuk projesi" diye adlandırılan araştırma 1963 yılında İngiltere'nin Cambridge kentindeki Tıp Araştırmaları Konseyi'nin Moleküler Biyoloji Laboratuvarı'nda başladı. Dr. **Sydney Brenner**'in başlattığı bu projeye 1970'li yıllarda Sir **John Sulston** ve Amerikalı Prof. **Robert Horwitz** katıldı.

Uzmanlar, bilimsel adı "caenorhabditis elegans" olan 1 milimetre boyundaki kurtçuğun üzerinde genlerin organ oluşumunu nasıl etkilediğini izlemeye aldılar. "C.elegans" diye adlandırılan ve 959 kadar hücresi olan kurtçukta, yumurta döneminden ölümüne kadar her bir hücrenin nasıl geliştiğini ve genlerin bu süreci nasıl etkilediğini takip ettiler.

Daha sonra kurtçuğun genleri deşifre edilmeye başlandı. Kurtçuk üzerindeki bu çalışmada kullanılan teknikten ileride insanın genetik kodunun çözülmesinde de faydanıldı. Araştırmanın bir sonraki aşamasında da genlerin hücrelere nasıl "intihar et" komutunu verdiğini belirlediler. Kurtçuğun belirli bir gelişme sağladığında bu genler, 959 hücreden 131'ine "ölüm emri" veriyordu.

Bu çalışmalarıyla Dr. **Sydney Brenner** (75), Sir **John Sulston** (60) ve Prof. **Robert Horvitz** (55), 1 milyon dolarlık Nobel tıp ödülünü paylaştılar.



AIDS İÇİN UMUT

Bilimadamları sadece insanın gen haritasının okunmasını sağlayan tekniğe öncülük ettikleri için değil, **"gen - organ gelişimi- hücre intiharı"** sistemi insanlarda da aynı olduğu için ödüle layık görüldüler. Uzmanlar ayrıca virüs ve bakterilerin hücreleri nasıl ele geçirdiklerini belirlediler. Araştırmada elde edilen veriler, Aids, felç, kalp krizi gibi hastalıklara karşı yeni tedavi yöntemlerinin geliştirilmesine olanak tanıyabilir.

Sir **John,** Nobel ödülü aldığını öğrenmesinin ardından **"Nobel'in gerçek sahibi C.elegans'tır."** dedi.

■ DIŞ HABERLER SERVİSİ

Apoptotic Cells in the Adult

Virtually all tissues harbor apoptotic cells at one time or another. The cells usually commit suicide for the greater good of the body. The list of examples here is far from exhaustive.

Eye. The lens of the eye, which forms during embryonic development, consists of apoptotic cells that have replaced their innards with the clear protein crystallin.

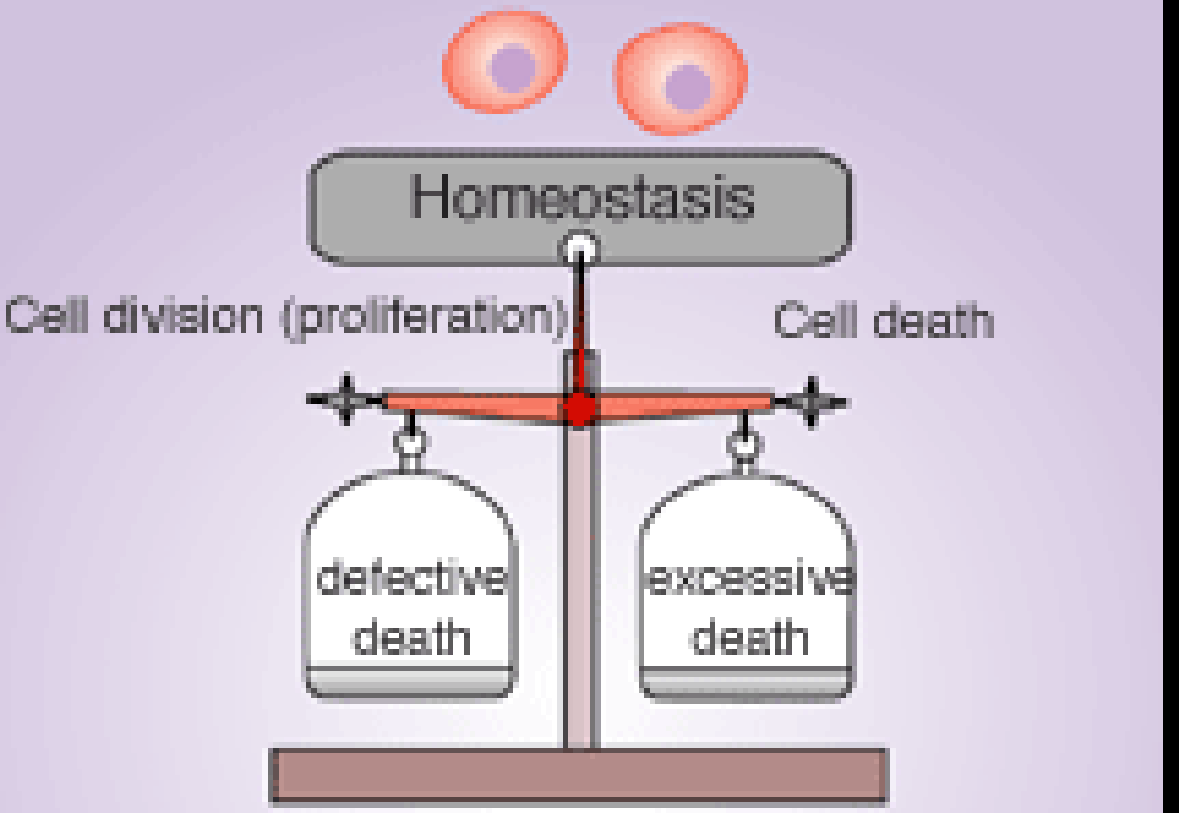
Intestine. Cells composing the fingerlike projections of the intestinal wall arise at the base of the "fingers" and, over several days, travel to the tip. They die there and are sloughed off.

Skin. Skin cells begin life in the deepest layers and then migrate to the surface, undergoing apoptosis along the way. The dead cells form the skin's protective outer layer.

Thymus. *T* lymphocytes—white blood cells that are critical components of the immune system—mature in the thymus. Those that would be ineffectual or would attack the body's own tissues commit suicide before they have the chance to enter the bloodstream.

Uterus. When the cells of the uterine wall die and are sloughed off during menstruation, they perish by apoptosis.

Other. Cells that become infected by a virus or sustain irreparable genetic mutations often kill themselves. Failure of a genetically altered cell to commit suicide can contribute to cancer.



Cancer
SLE
Rheumatoid arthritis
Polycythemia vera

Huntington's disease
ALS
Shigellosis
AIDS
Stroke
Myocardial infarction

Apoptozisin İnhibisyonu İle İlgili Hastalıklar

- **Kanser**
  - **Foliküler lenfoma**
  - **P53 mutasyonlu karsinomlar**
  - **Hormon-bağımlı tümörler**
    - **Meme Ca**
    - **Prostat Ca**
    - **Over Ca**

- **Viral enfeksiyonlar**
  - **Herpes viruslar**
  - **Pox viruslar**
  - **Adenoviruslar**

- **Otoimmün bozukluklar**
  - **SLE**
  - **İmmün-aracılı GN**

Apoptozisin Artışı İle İlgili Hastalıklar

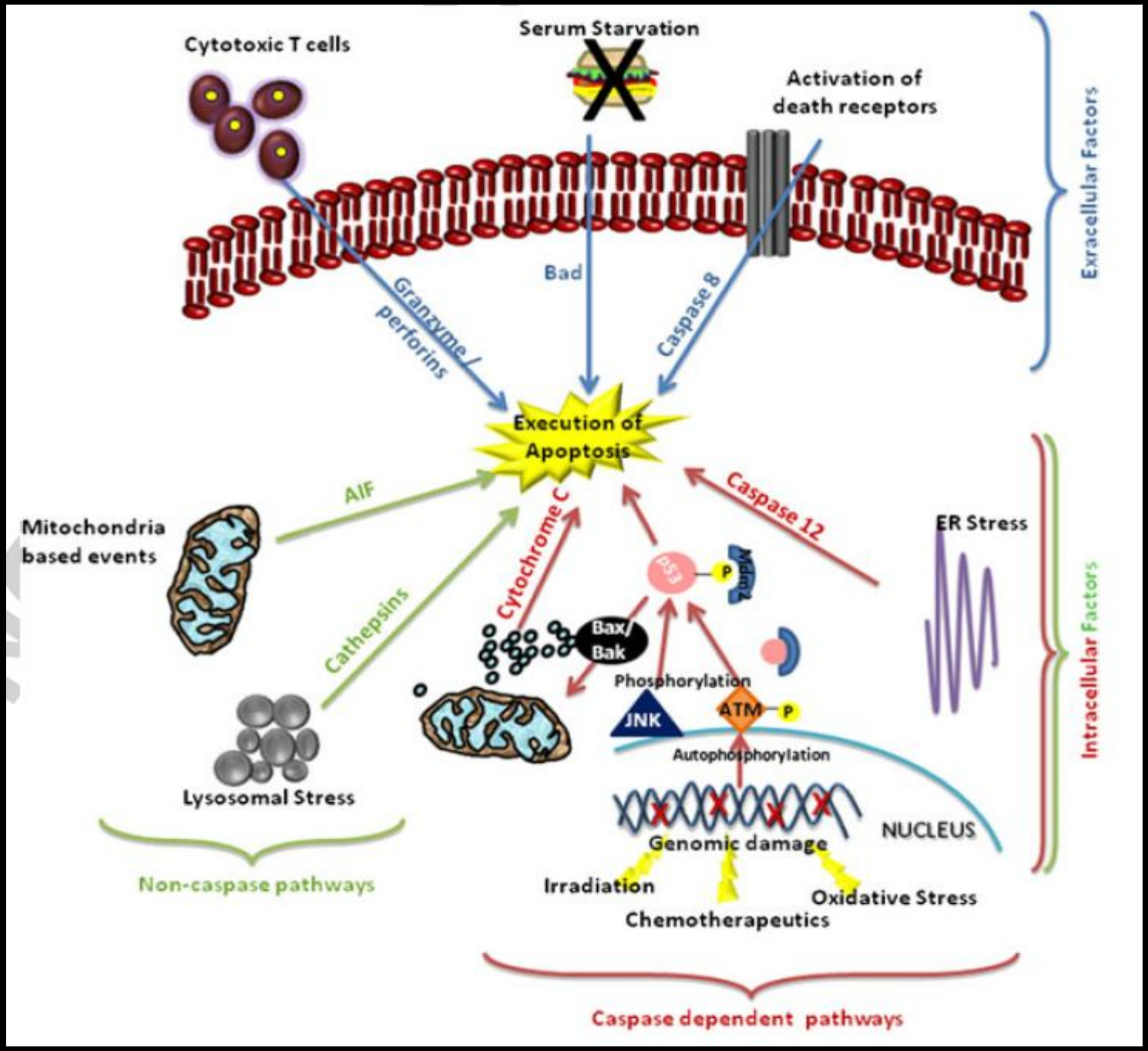
- **AIDS**

- **İskemik hasar**
  - **MI**
  - **Stroke**
  - **Reperfüzyon hasarı**

- **Nörodejeneratif bozukluklar**
  - **Alzheimer hastalığı**
  - **Parkinson hastalığı**
  - **Retinitis pigmentosa**
  - **Serebellar dejenerasyon**

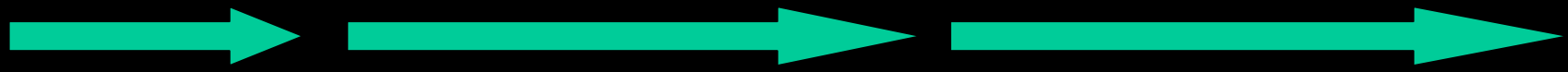
- **Miyelodisplastik Sendromlar**
  - **MM**

- **Toksinle indüklenen karaciğer hasarı**
  - **Alkol**



Ulukaya et al, *Cell Biochem Func,* 2011

THE CELL UNDERGOING APOPTOSIS



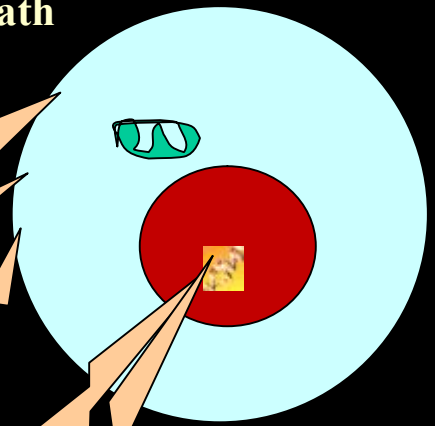
1. Activation of death receptors
. Fas
. TNFR1
. DR4, DR5

2. Granzymes

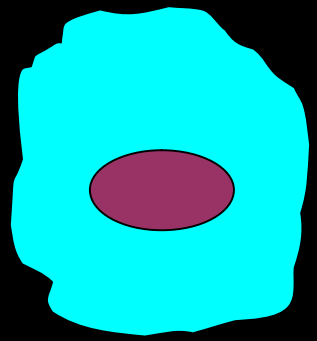
3. Lack of survival factors

4. Gene damag
. Radiation
. Chemotherapy

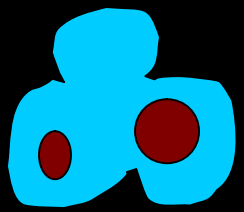
5. Shortening of telomers



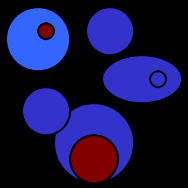
. FADD
. TRADD
. FLIP
. Bcl-2 family
. Cytochrom *c*
. p53
. Mdm2
. IAP group (cIAP1, cIAP2, XIAP ve Survivin)



. Caspases



. Many cellular proteins
. DNA



. Apoptotic bodies
. Phagocytosis

Ulukaya *et al.*,

SLE ve Apoptozis

- **Apoptotik hücrelerin temizlenmesi defektif**
- **Fosfotidil-serin'in (PS) eksternalizasyonu ile ilişkili olarak antifosfolipid Ab'ların yapımı**

RA ve Apoptozis

- **RA'lhastalarda Fibroblast-benzeri sinoviositlerde (FLS) ve T hücrelerinde Bcl-2, Bcl-xL ve survivin ekspresyonu ⬆ (Apopitozisi inh.ederler)**
  - **Bu hücrelerde sinoviyal hiperplazi**

Grodzicky and Elkon, Mount Sinai J Med, 2002

Otoimmün Diabet ve Apoptozis

- **Hem $CD4^+$ hem de $CD8^+$ T lenfositlerin, pankreatik β hücrelerini hasarlaması (perforin bağımlı sitotoksisite)**

- **Fas/FasL aracılı mekanizmalarla pankreatik β hücrelerinin *hasarı***

Stalder et al.. J Immunol, 1994

Kagi et al.. J Exp Med, 1996

Yang et al.. J Exp Med, 1994

MS ve Apoptozis

- **Aktif veya kronik sessiz m. sklerozlulardan alınan örneklerde ise Fas ve FasL'ın aşırı ekspresyonu saptanmış.**

Bonetti et al.. Ann Neurol, 1997
Dowling et al. J Exp Med 1996

HIV enfeksiyonu ve Apoptozis

- **HIV *nef* geninin enfekte hücrelerde ekspresyonu, bu hücrelerde FasL ekspresyonunu arttırırken Fas ekspresyonunu düşürür**

- **Enfekte hücreler enfekte olmayan hücrelerle karşılaştığında bunları Fas/FasL etkileşimi sonucunda apoptozise uğratır.**

Alimonti et al.. J Gen Virol, 2003

Parkinson Hastalığı ve Apoptozis

- Postmortem olarak dopaminerjik nöronlarda p53, Fas, TNF-R ve kaspazların (kaspaz 3, 8 ve 9) ekspresyonlarının arttığı gösterilmiştir.

Alzheimer ve Apoptozis

- Alzheimer hastalarından alınan ölen nöronlarda aktif kaspazlar saptanmıştır. Bu enzimler β-amiloidi katalize ederek pro-apoptotik protein oluşmasına yol açabilmektedir.

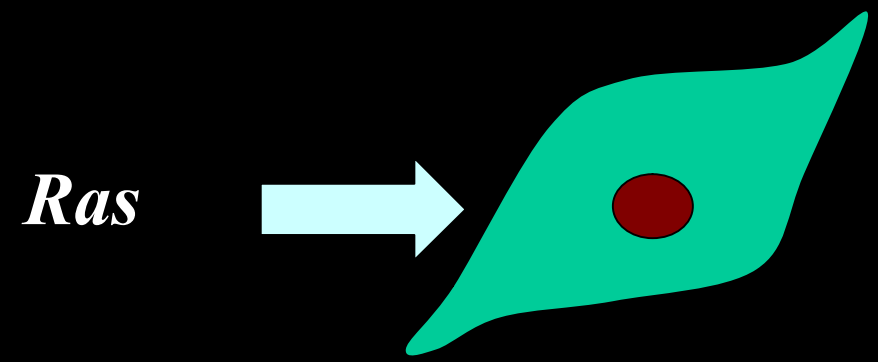
- β-amiloid nöron kültürlerinde apoptozisi indükleyebilir.

Kanser ve Apoptozis

APOPTOZİS 

KANSER 

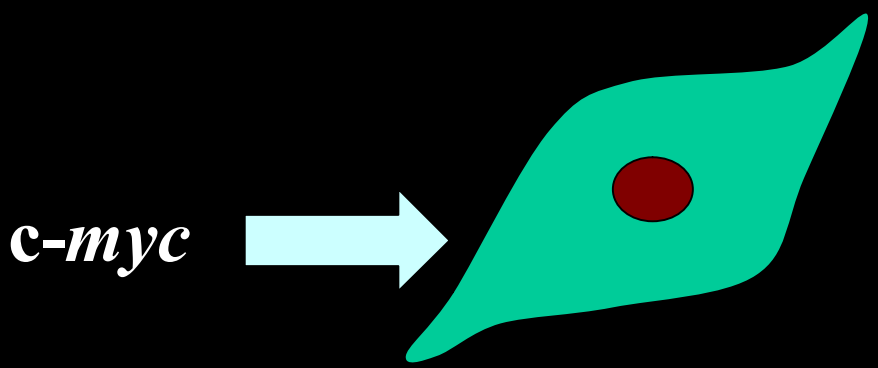
Transfection studies in rat fibroblasts



Ras

Apoptosis ↓

Tumor growth ↑



c-*myc*

Apoptosis ↑

Tumor growth ↓

Kanser hücreleri

ölmeyi unuturlar !☺

Tümör Hücrelerinin Apoptozisden Kurtulma Mekanizmaları

- **Fas ve Fas Ligand (Fas-L) interaksiyonları**
- **“Decoy” reseptörlerinin varlığı**
- **p53 mutasyonu**
- **Aşırı Bcl-2 ekspresyonu ve/veya azalmış Bax ekspresyonu**
- **FLIP aşırı ekspresyonu**
- **Kaspaz inhibitörlerinin aşırı ekspresyonları (cIAP2, Survivin)**

THE CELL UNDERGOING APOPTOSIS

1. Activation of death receptors
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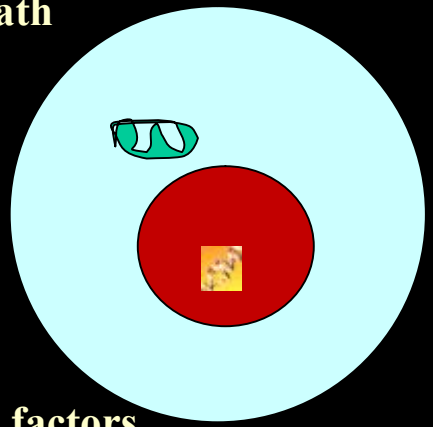

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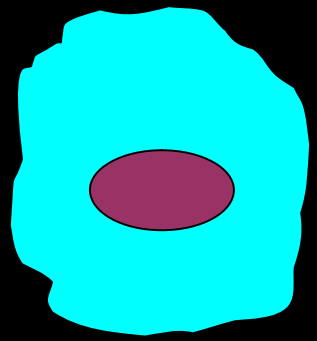

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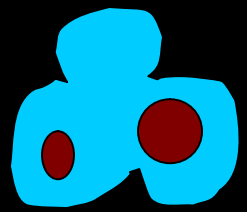

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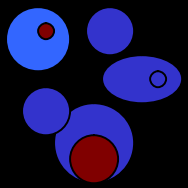

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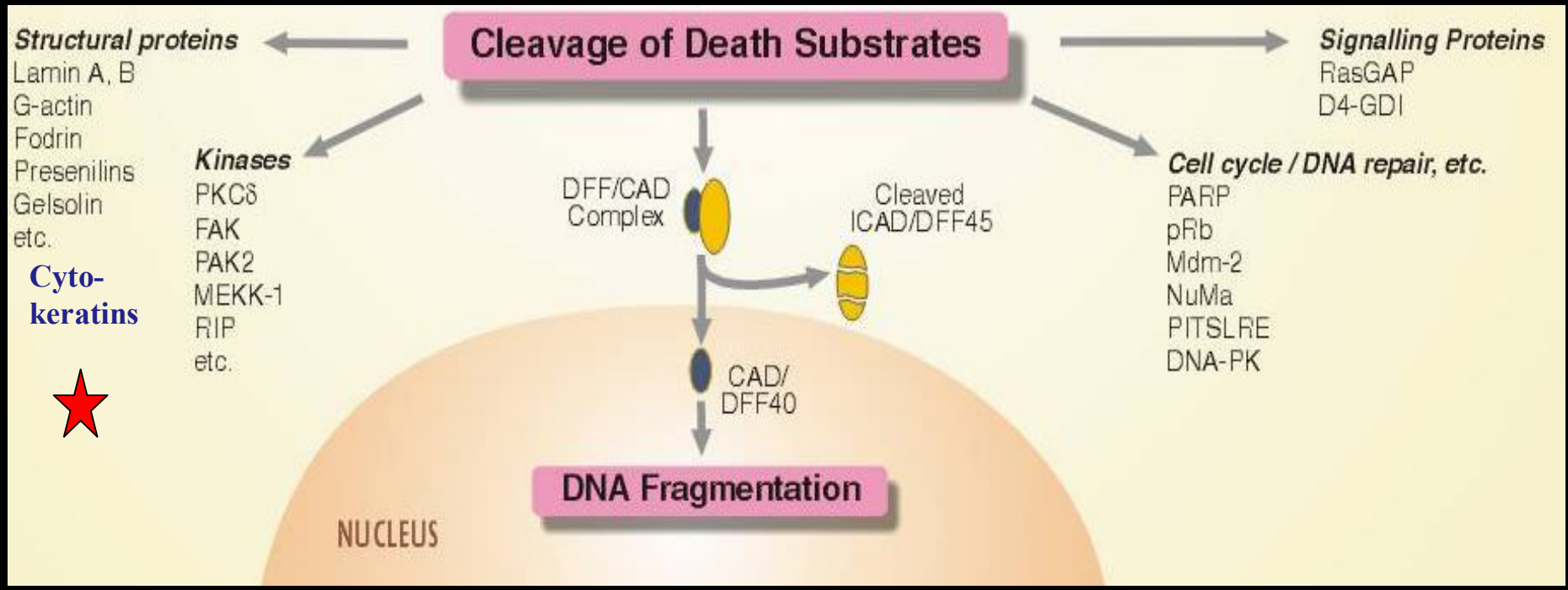
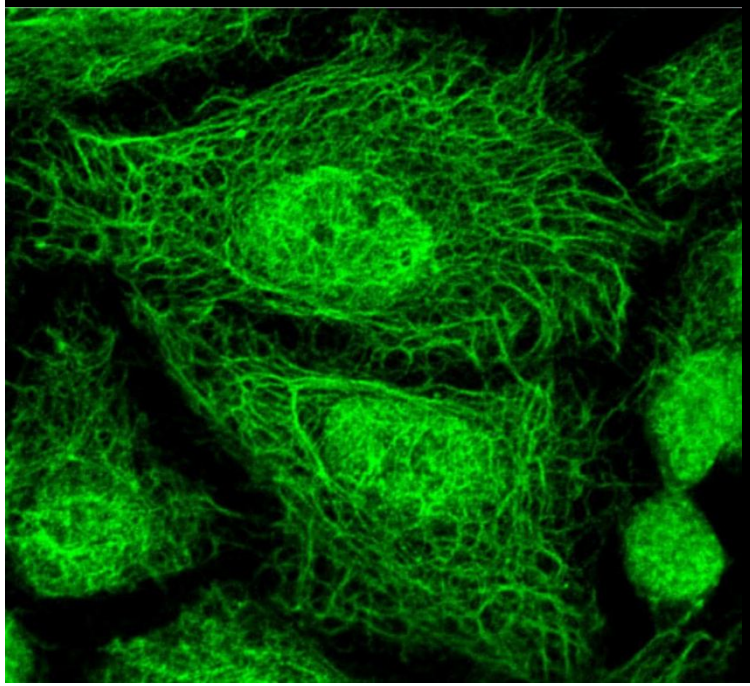
Dr. E Ulukaya

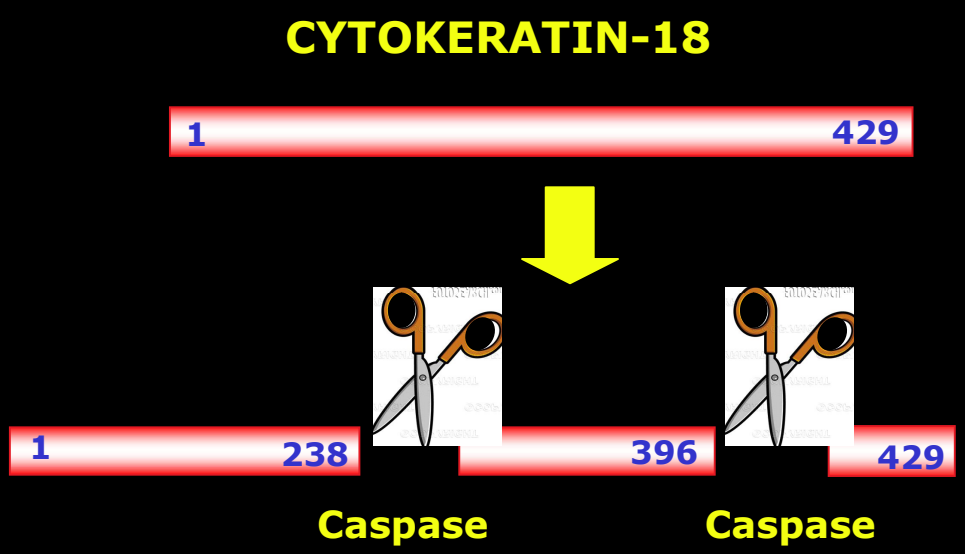

Cleavage of Death Substrates
Structural proteins
Lamin A, B
G-actin
Fodrin
Presenilins
Gelsolin
etc.
Cyto-keratins
Kinases
PKCδ
FAK
PAK2
MEKK-1
RIP
etc.
DFF/CAD Complex
Cleaved ICAD/DFF45
CAD/ DFF40
DNA Fragmentation
NUCLEUS
Signalling Proteins
RasGAP
D4-GDI
Cell cycle / DNA repair, etc.
PARP
pRb
Mdm-2
NuMa
PITSLRE
DNA-PK

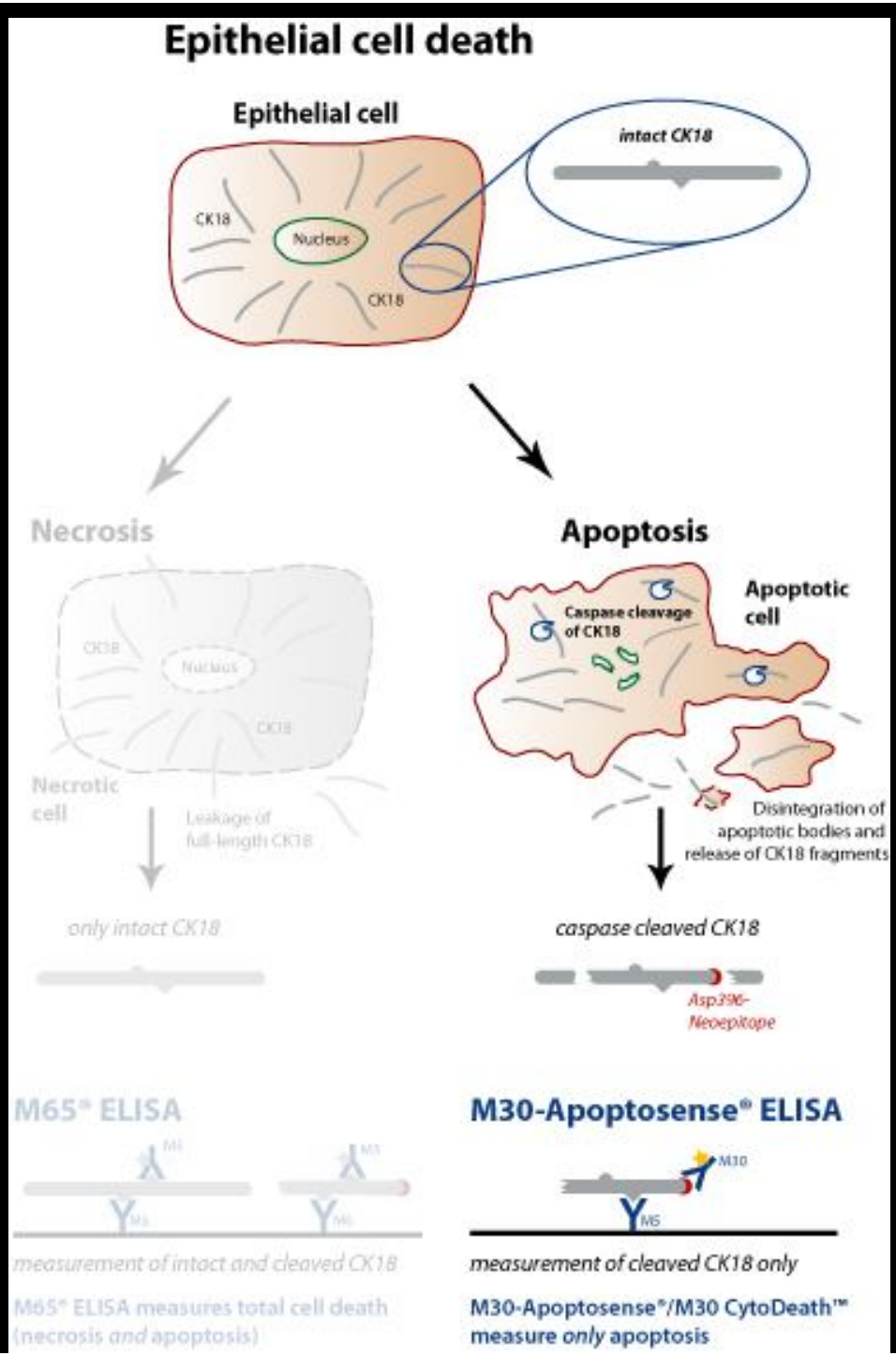
CYTOKERATIN-18

A CELL DEATH PLASMA BIOMARKER FOR EPITHELIAL CELLS

1. INTRACELLULAR; STRONG EXPRESSION
2. EPITHELIAL SPECIFIC PROTEIN (not expressed by treatment-sensitive cells in bone marrow etc.)
3. CLEAVED BY CASPASES DURING APOPTOSIS



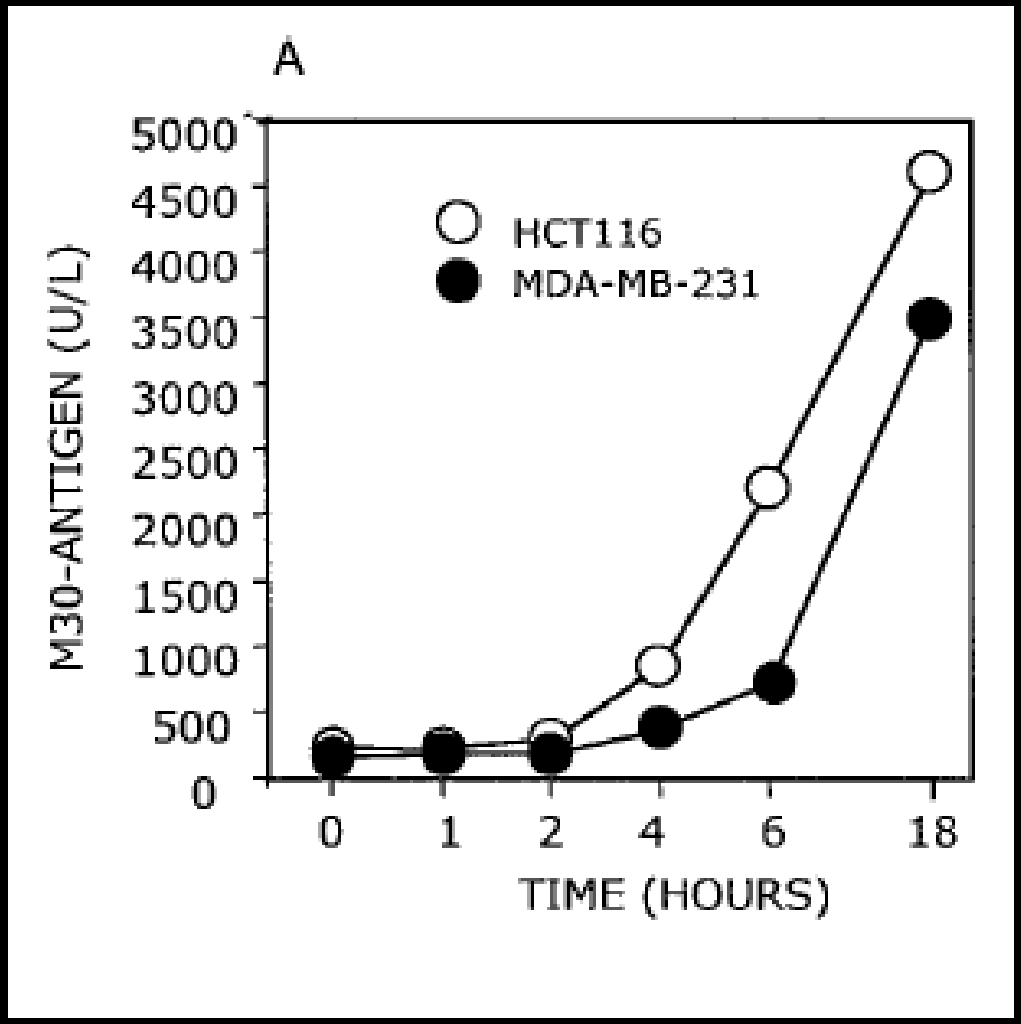



Epithelial cell death
Epithelial cell
intact CK18
CK18
Nucleus
CK18
Necrosis
Apoptosis
Apoptotic cell
Caspase cleavage of CK18
Nucleus
CK18
Necrotic cell
Leakage of full-length CK18
Disintegration of apoptotic bodies and release of CK18 fragments
only intact CK18
caspase cleaved CK18
Asp396-Neoepitope
M65® ELISA
M30-Apoptosense® ELISA
M30
measurement of intact and cleaved CK18
measurement of cleaved CK18 only
M65® ELISA measures total cell death (necrosis and apoptosis)
M30-Apoptosense®/M30 CytoDeath™ measure only apoptosis

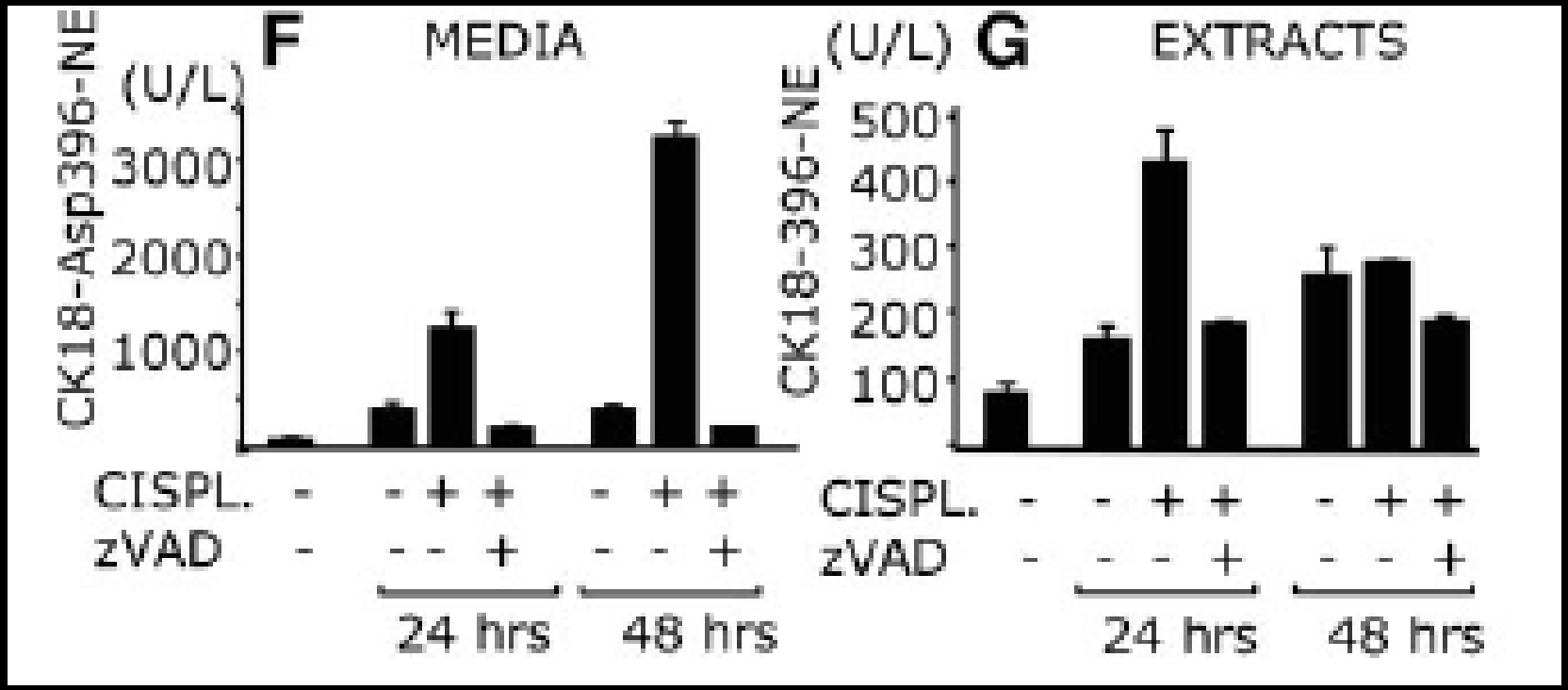
- M30 ELISA Assay is a useful tool for discovery of pro-apoptotic drugs

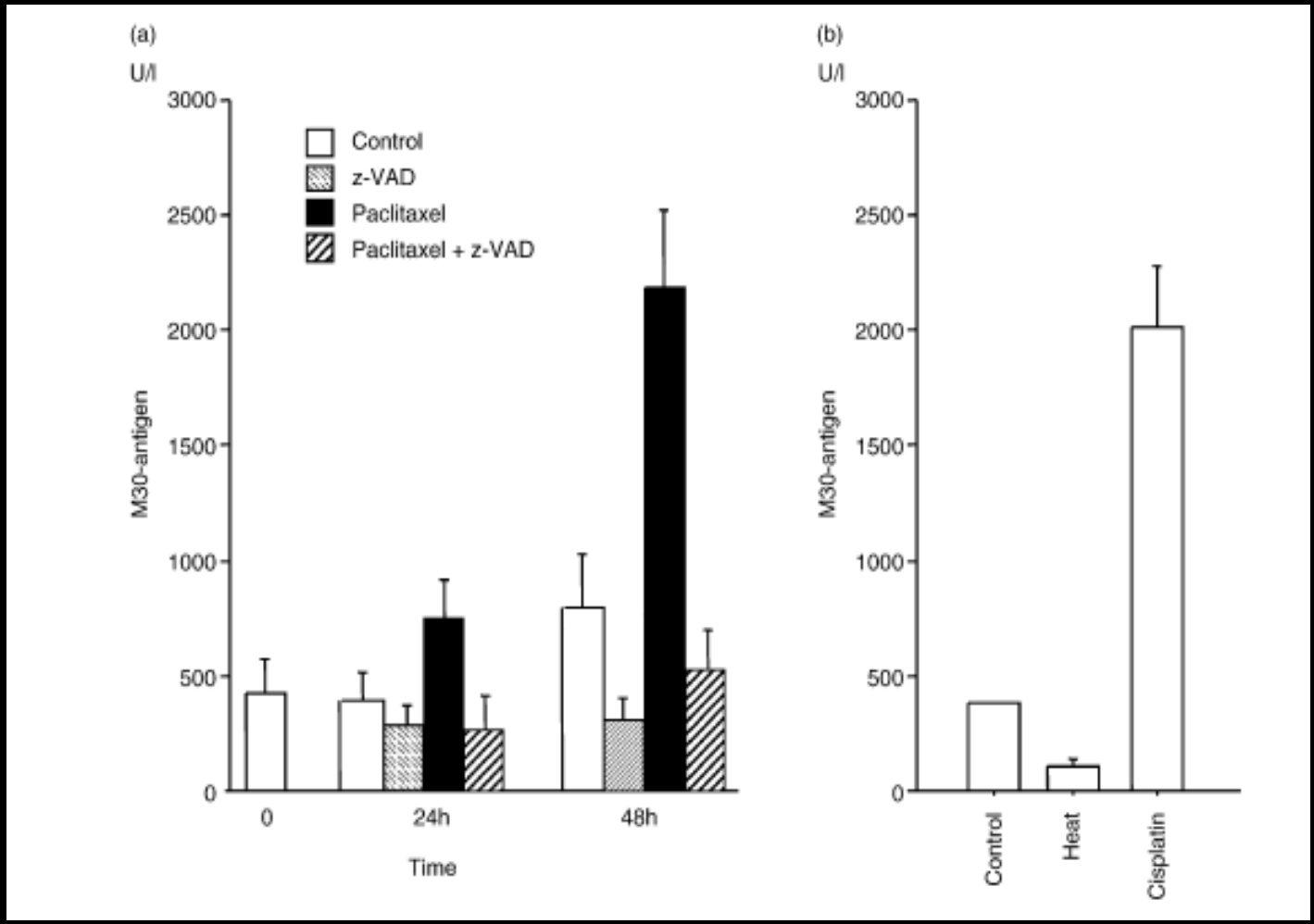
Hagg et al, Investigational New Drugs, 2002

Biven K, Apoptosis, 2003



Kramer G, Cancer Res, 2004




(a)
U/l
3000
2500
2000
1500
1000
500
0
M30-antigen
Control
z-VAD
Paclitaxel
Paclitaxel + z-VAD
0
24h
48h
Time
(b)
U/l
3000
2500
2000
1500
1000
500
0
M30-antigen
Control
Heat
Cisplatin

TRANSLATIONAL STUDIES:
TESTING DRUG EFFECTS IN ANIMALS AND PATIENTS

CASPASE-CLEAVED CYTOKERATIN-18 AS A BLOOD PHARMACODYNAMIC MARKER FOR DRUG-INDUCED TUMOR APOPTOSIS

XENOGRAFT

CLINICAL STUDIES

TRANSLATIONAL STUDIES:
TESTING DRUG EFFECTS IN ANIMALS

XENOGRAFT

CASPASE-CLEAVED CK18 IN PLASMA FROM XENOGRAFT RODENT MODELS

Preclinical evaluation of M30 and M65 ELISAs as biomarkers of drug induced tumor cell death and antitumor activity

Jeffrey Cummings,[1] Cassandra Hodgkinson,[1] Rajesh Odedra,[2] Patrizia Sini,[2] Simon P. Heaton,[2] Kirsten E. Mundt,[2] Tim H. Ward,[1] Robert W. Wilkinson,[2] Jim Growcott,[2] Andrew Hughes,[2] and Caroline Dive[1]

MOL CANCER THERAPEUTICS 7, 455, 2008

Cancer Therapy: Preclinical

Circulating Biomarkers of Cell Death After Treatment with the BH-3 Mimetic ABT-737 in a Preclinical Model of Small-Cell Lung Cancer

Dimitra Micha,[1] Jeff Cummings,[1] Alex Shoemaker,[2] Steven Elmore,[2] Kelly Foster,[2] Martin Greaves,[1] Tim Ward,[1] Saul Rosenberg,[2] Caroline Dive,[1] and Kathryn Simpson[1]

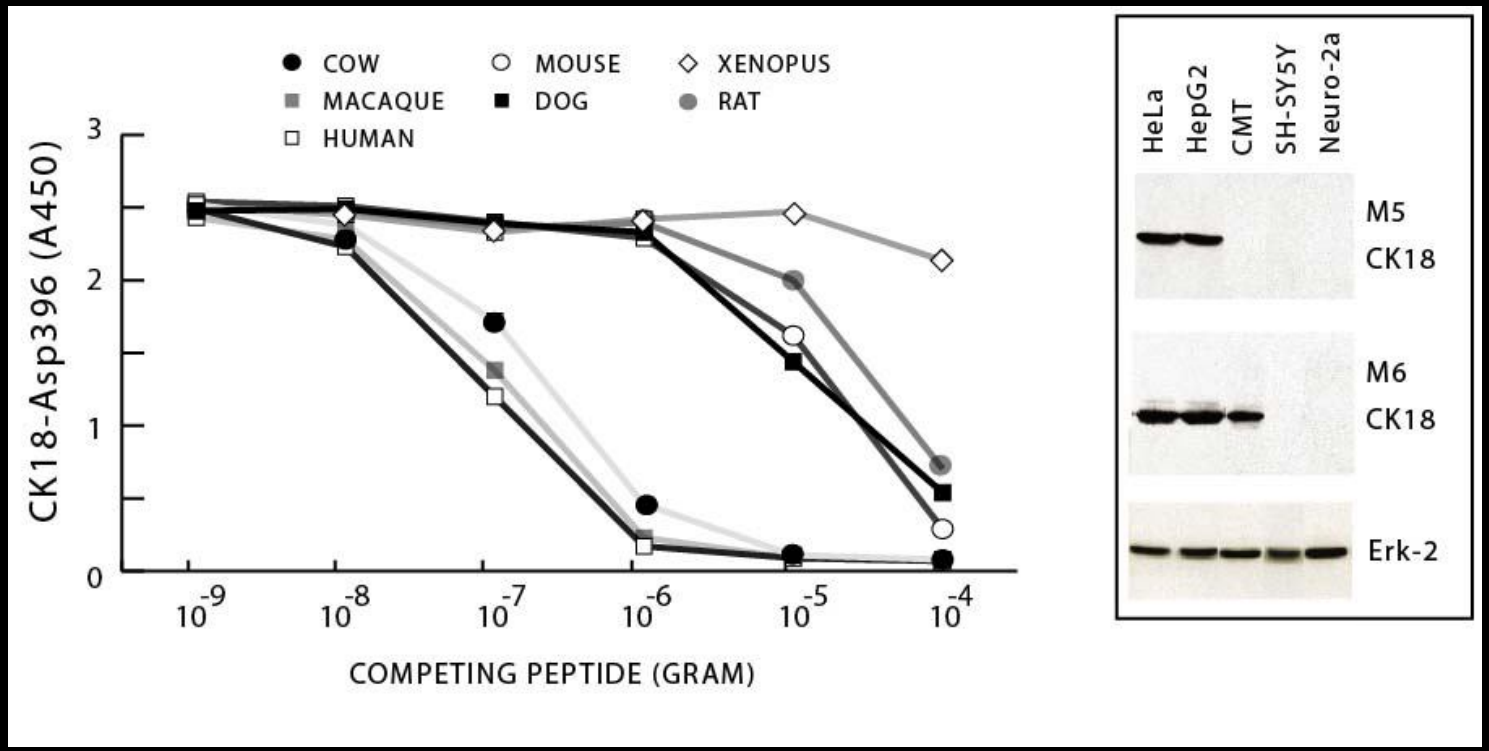
CLIN CANCER RES 14, 7304, 2008

Specific demonstration of drug-induced tumour cell apoptosis in human xenografts models using a plasma biomarker

M. Hägg Olofsson[a], J. Cummings[b], W. Fayad[a], S. Brnjic[a], R. Herrmann[a], M. Berndtsson[a], C. Hodgkinson[b], E. Dean[b], R. Odedra[c], R.W. Wilkinson[c], K.E. Mundt[c], M. Busk[d], C. Dive[b] and S. Linder[a,*]

CANCER BIOMARKERS 5, 117, 2009

M30 AND M65 ELISA ASSAYS RECOGNIZE HUMAN - BUT NOT MOUSE - CK18



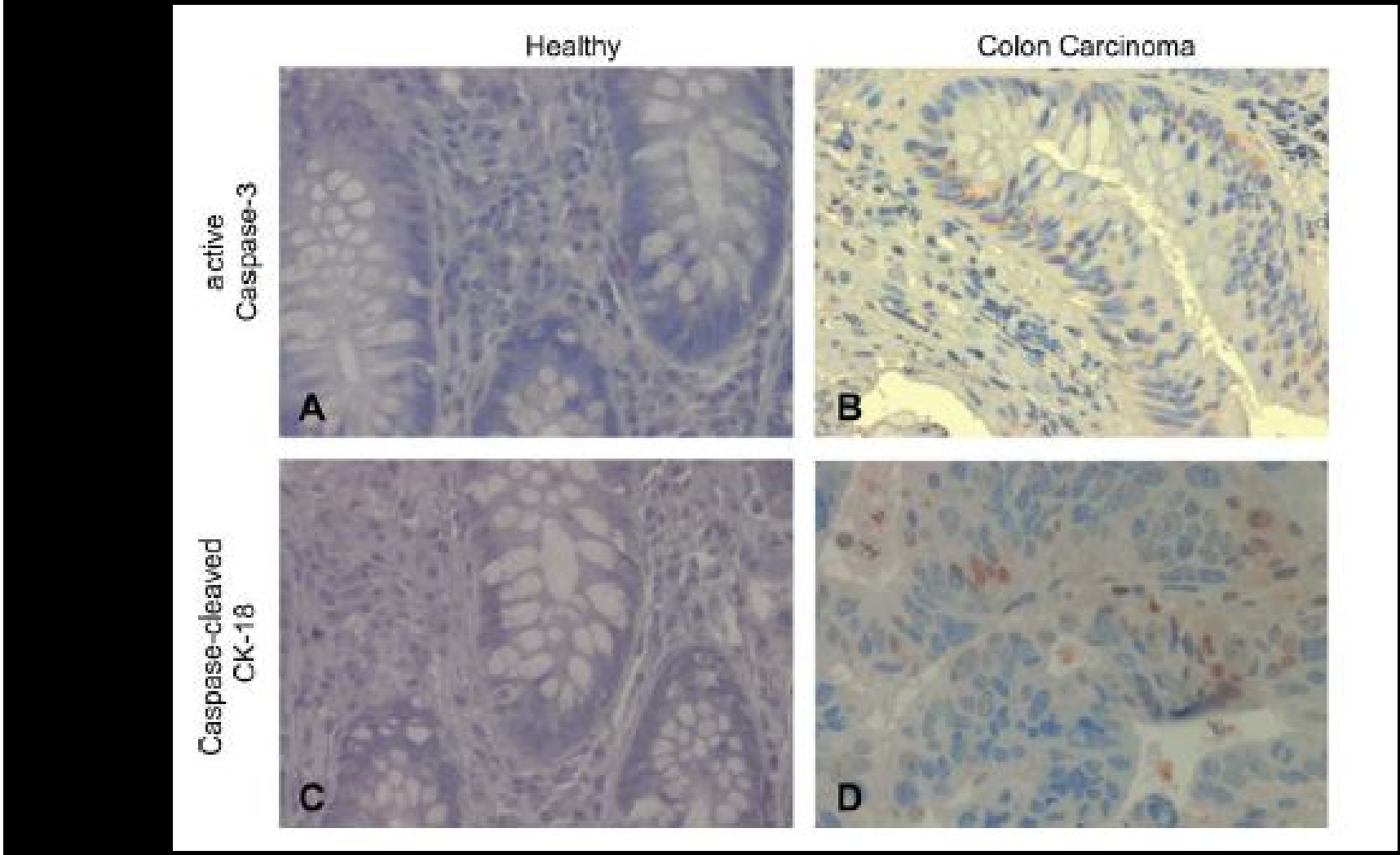
HUMAN XENOGRAFTS IN SCID MICE: DETERMINE TUMOR SPECIFIC APOPTOSIS IN PLASMA

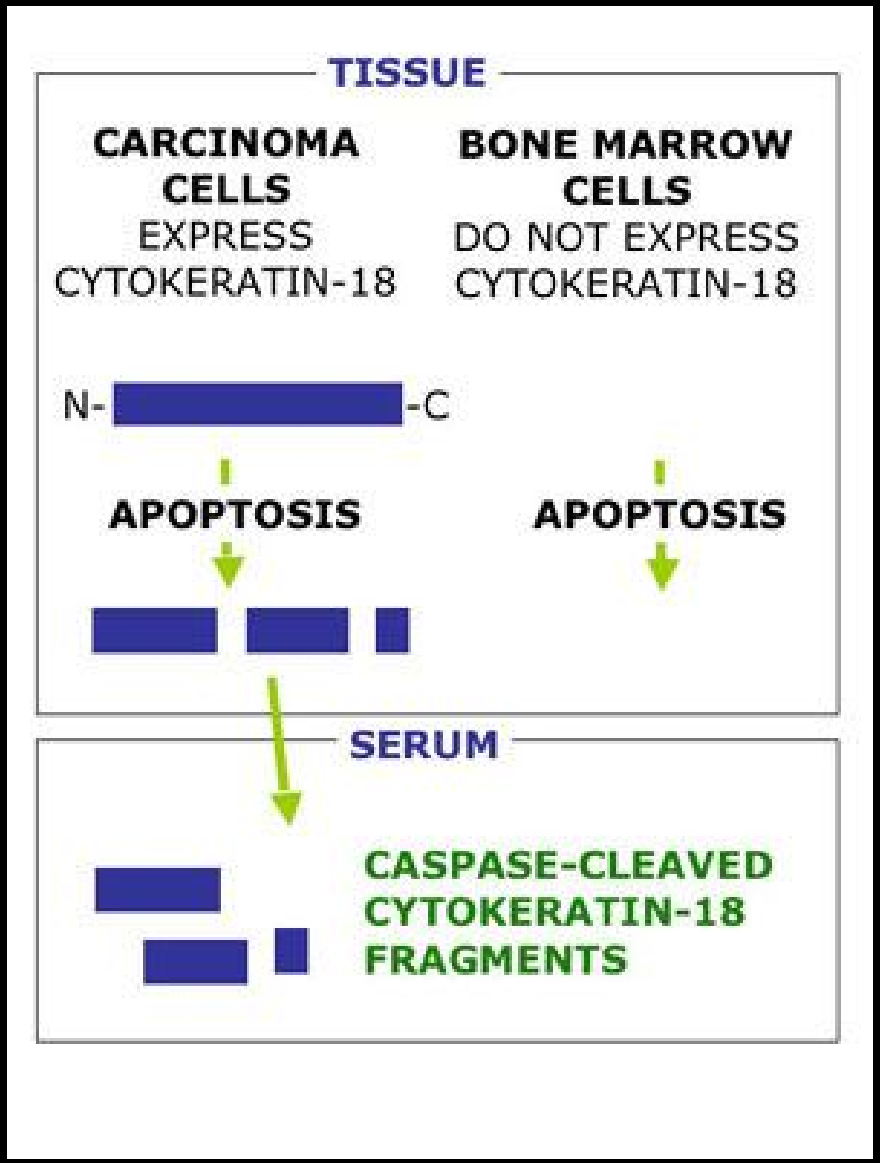
TRANSLATIONAL STUDIES:
TESTING DRUG EFFECTS IN PATIENTS

CLINICAL STUDIES

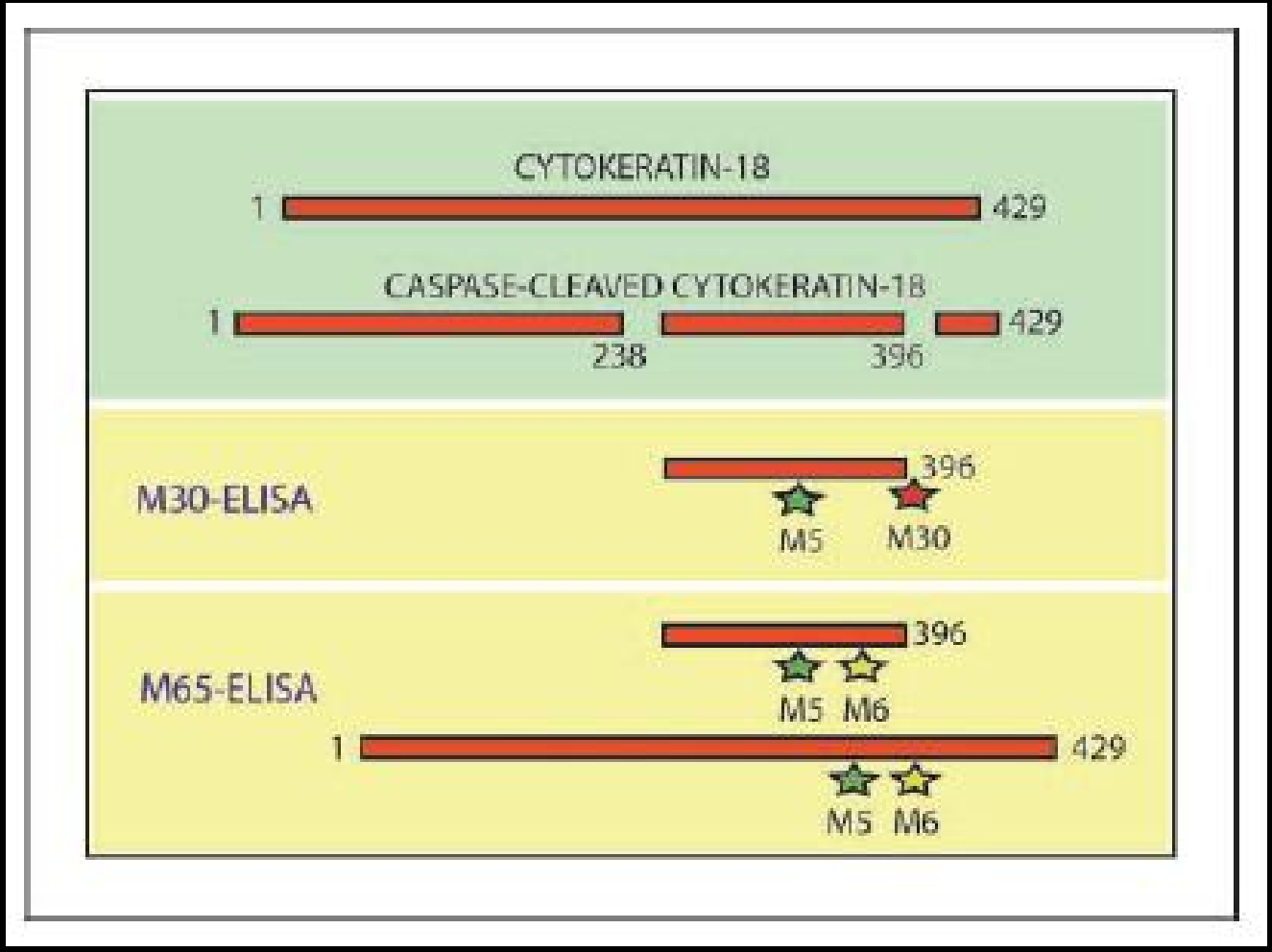
Apoptozis Araştırmalarının Hastaya Yansıması

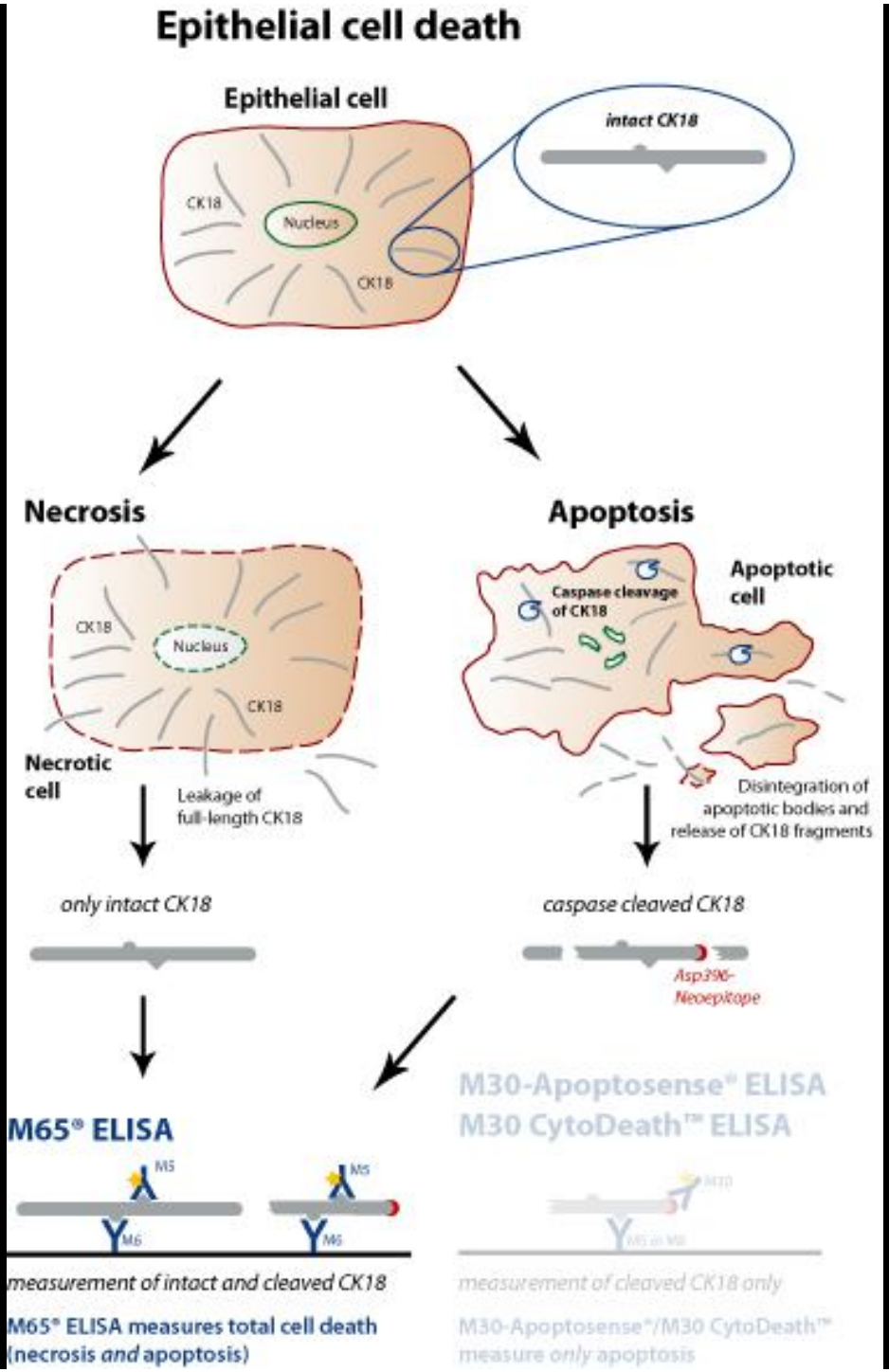
(Translational Research in Apoptosis)


Healthy
Colon Carcinoma
active Caspase-3
Caspase-cleaved CK-18
A
B
C
D

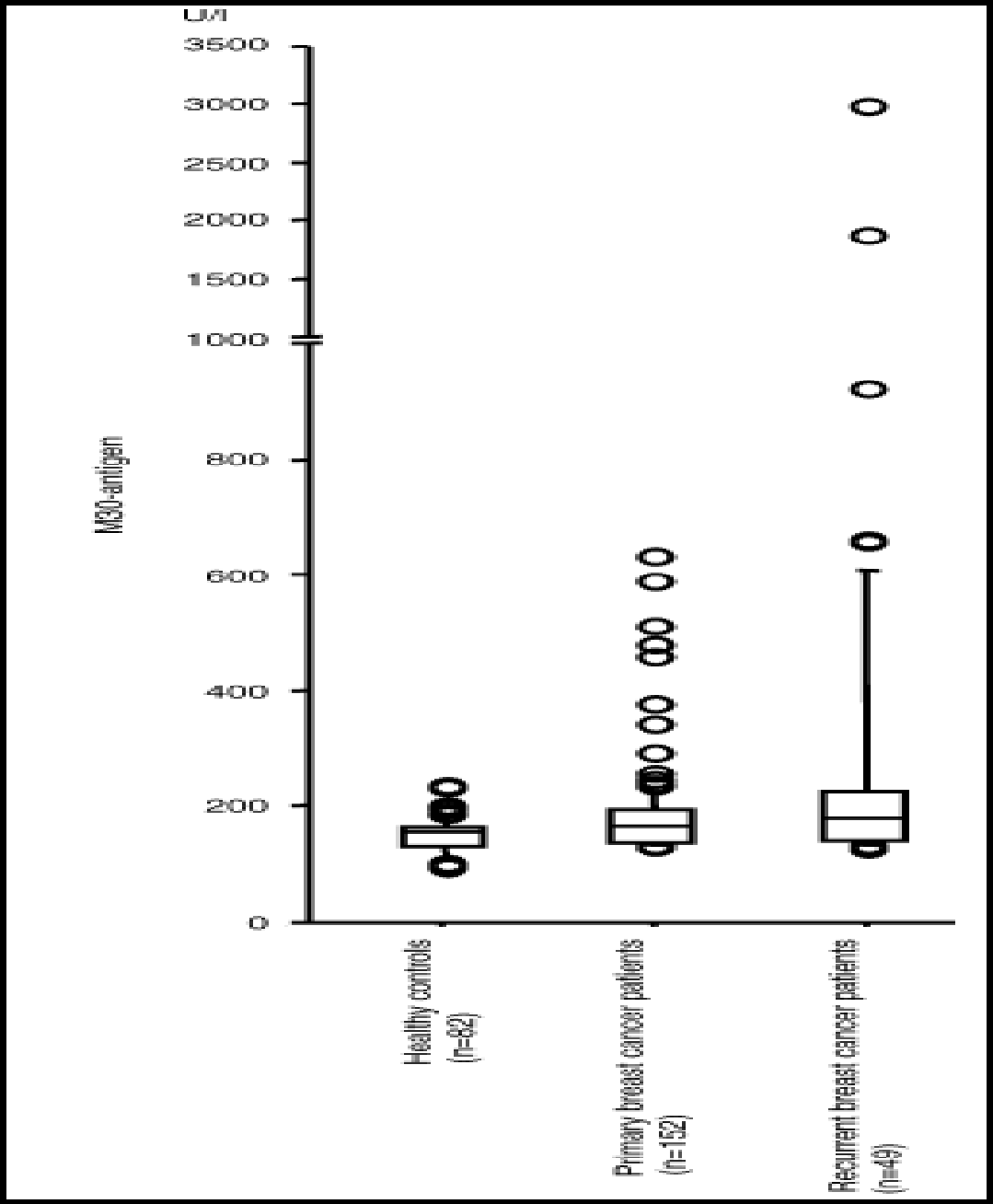

TISSUE
CARCINOMA CELLS
EXPRESS
CYTOKERATIN-18
BONE MARROW CELLS
DO NOT EXPRESS
CYTOKERATIN-18
N-
-C
APOPTOSIS
APOPTOSIS
SERUM
CASPASE-CLEAVED
CYTOKERATIN-18
FRAGMENTS

Olofsson HM, Clin Cancer Res, 2007




Epithelial cell death
Epithelial cell
intact CK18
CK18
Nucleus
CK18
Necrosis
Apoptosis
CK18
Nucleus
CK18
Necrotic cell
Leakage of full-length CK18
Caspase cleavage of CK18
Apoptotic cell
Disintegration of apoptotic bodies and release of CK18 fragments
only intact CK18
caspase cleaved CK18
Asp396-Neoepitope
M65® ELISA
M5
M5
M6
M6
measurement of intact and cleaved CK18
M65® ELISA measures total cell death (necrosis and apoptosis)
M30-Apoptosense® ELISA
M30 CytoDeath™ ELISA
M30
measurement of cleaved CK18 only
M30-Apoptosense®/M30 CytoDeath™ measure only apoptosis

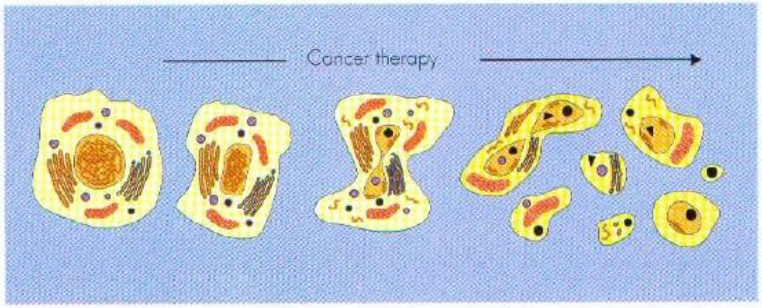
Ueno T, Euro J Cancer, 2003



Kaspazla kırılmış sitokeratin18 (M30 antijen):

Acaba kemoterapiye yanıtı belirlemede kullanılabilir mi?

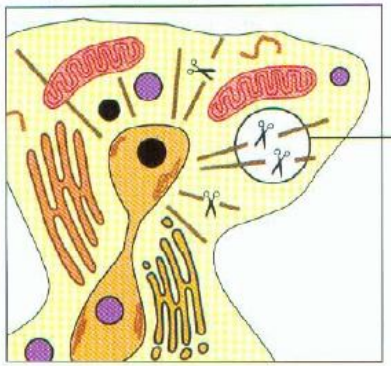
CANCER THERAPY INDUCES APOPTOSIS OF TUMOR CELLS



Apoptosis is an active process, resulting in degradation of cell constituents and fragmentation of the cell.

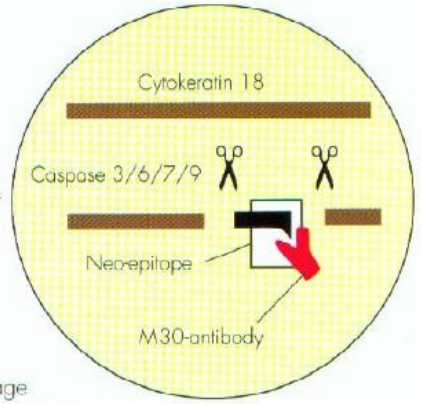
A family of proteases called *caspases* are responsible for a major part of this process.

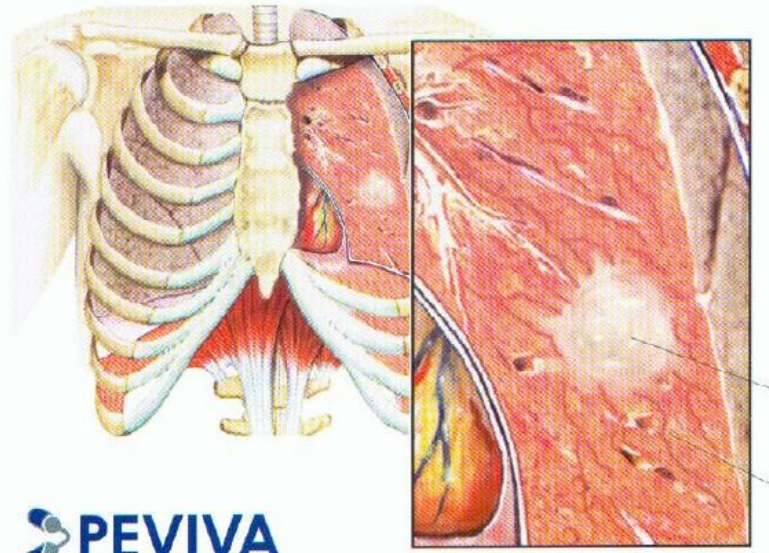
In epithelial cells, the cytoskeleton is degraded by caspases.



The filament protein cytokeratin 18 is cleaved at two sites.

Antibody M30 recognizes a neo-epitope of cytokeratin 18 which becomes exposed after cleavage by caspases during apoptosis.



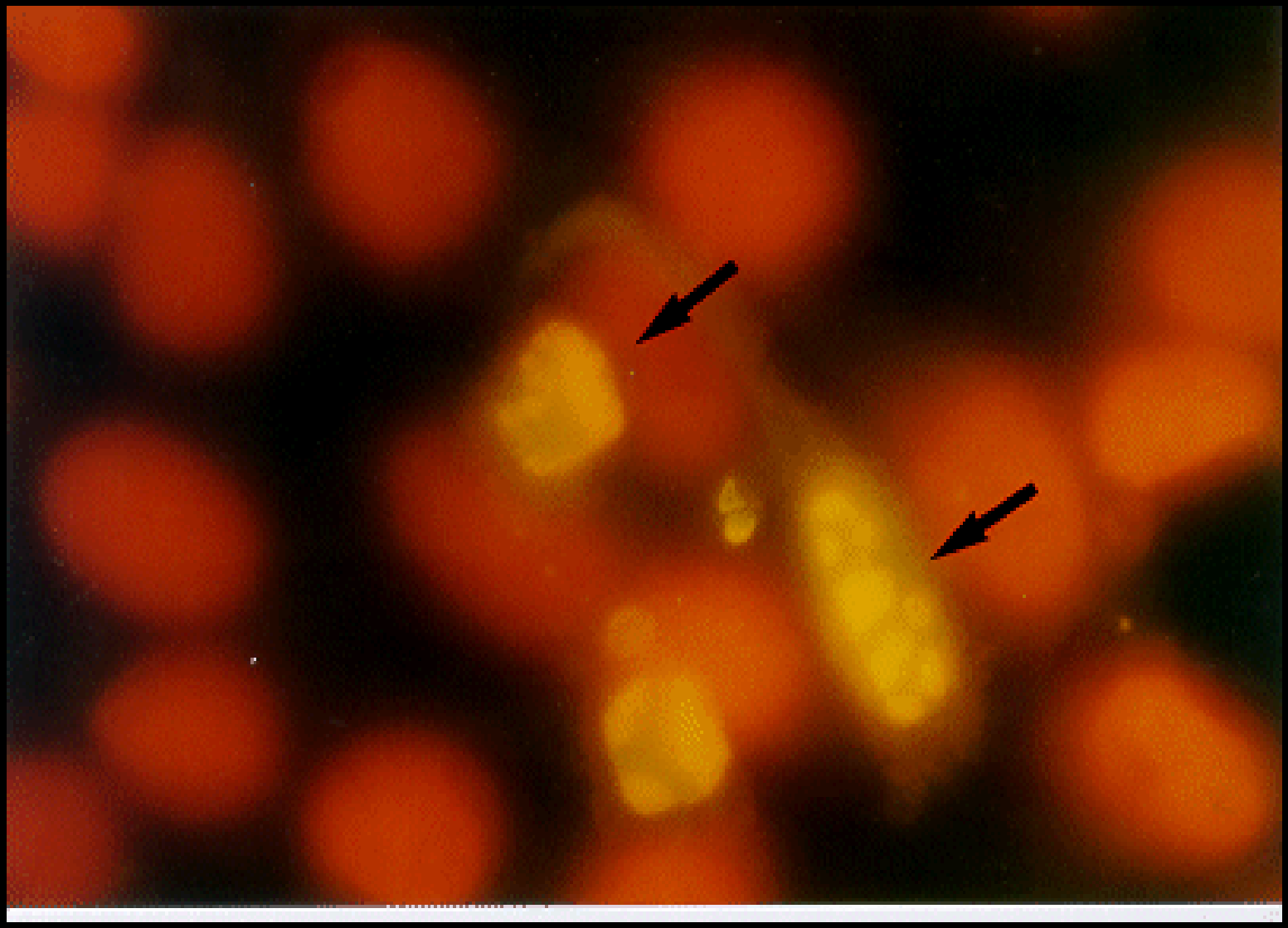


Caspase-cleaved cytokeratin 18 fragments are released from tumor cells during apoptosis. Of these fragments, the neo-epitope can be measured in sera from cancer patients by an ELISA-assay based on the M30-antibody.

Tumor

Release of caspase-cleaved cytokeratin 18 fragments into blood.

PEVIVA



Cancer Investigation, 24:669–676, 2006
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.1080/07357900600981307

informa
healthcare

ORIGINAL ARTICLE

Response to Neoadjuvant Chemotherapy in Breast Cancer Could be Predictable by Measuring a Novel Serum Apoptosis Product, Caspase-Cleaved Cytokeratin 18: A Prospective Pilot Study

Mutlu Demiray, M.D.,[1] Engin Ulukaya, M.D., Ph.D.,[2] Murat Arslan, M.D.,[1] Sehsuvar Gokgoz, M.D.,[3] Ozlem Saraydaroglu, M.D.,[4] Ilker Ercan, Ph.D.,[5] Turkkan Evrensel, M.D.,[1] and **Osman Manavoglu, M.D.[1]**

Uludag University Medical School, Department of Medical Oncology, Gorukle/Bursa, Turkey.[1]
Uludag University Medical School, Department of Biochemistry, Gorukle/Bursa, Turkey.[2]
Uludag University Medical School, Department of General Surgery, Gorukle/Bursa, Turkey.[3]
Uludag University Medical School, Department of Pathology, Gorukle/Bursa, Turkey.[4]
Uludag University Medical School, Department of Biostatistics, Gorukle/Bursa, Turkey.[5]

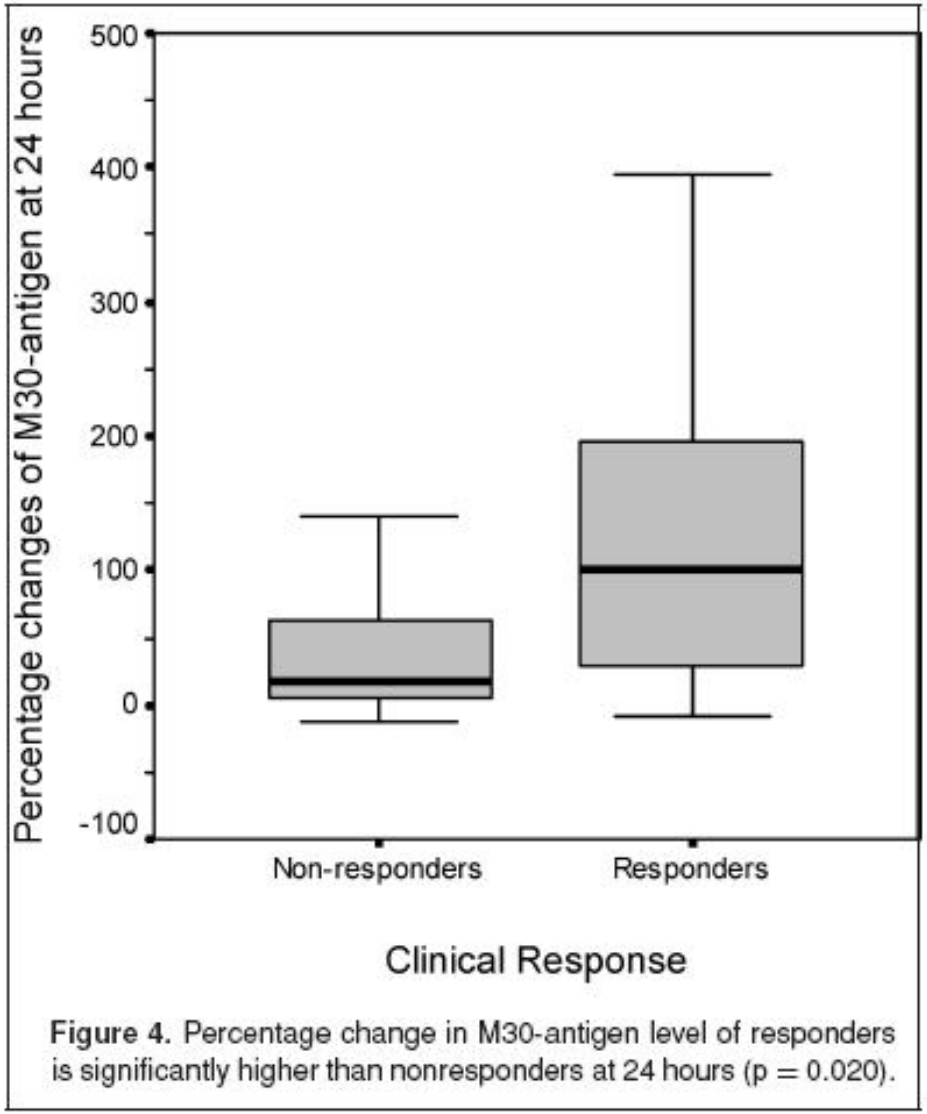


Figure 4. Percentage change in M30-antigen level of responders is significantly higher than nonresponders at 24 hours ($p = 0.020$).

Lung Cancer (2007) 56, 399–404

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan

ELSEVIER

LUNG CANCER

The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival

Engin Ulukaya[a,*], Arzu Yilmaztepe[a], Semra Akgoz[b], Stig Linder[c], Mehmet Karadag[d]

[a] *Biochemistry Department of Medical School of Uludag University, Bursa, Turkey*
[b] *Biostatistics Department of Medical School of Uludag University, Bursa, Turkey*
[c] *Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden*
[d] *Chest Disease and Tuberculosis Department of Medical School of Uludag University, Bursa, Turkey*

Ulukaya E, Lung Cancer, 2007

Table 3 Increments in M30 antigen levels after chemotherapy

M30 antigen level (U/L)	Before chemotherapy (n = 18)	24 h after chemotherapy (n = 18)	48 h after chemotherapy (n = 18)
Mean (±S.D.)	97.35 (±68.30)	165.73 (±120.23)	376.97 (±570.26)
(Minimum–maximum)	(4.67–268.02)	(3.89–454.59)	(30.53–2251.91)
Median [interquartile range]	102.91 [31.32–143.65]	138.39 [88.50–215.18]	177.37 [56.73–363.42]
p-Value		0.002*	0.001**

Cisplatin-based chemotherapy!

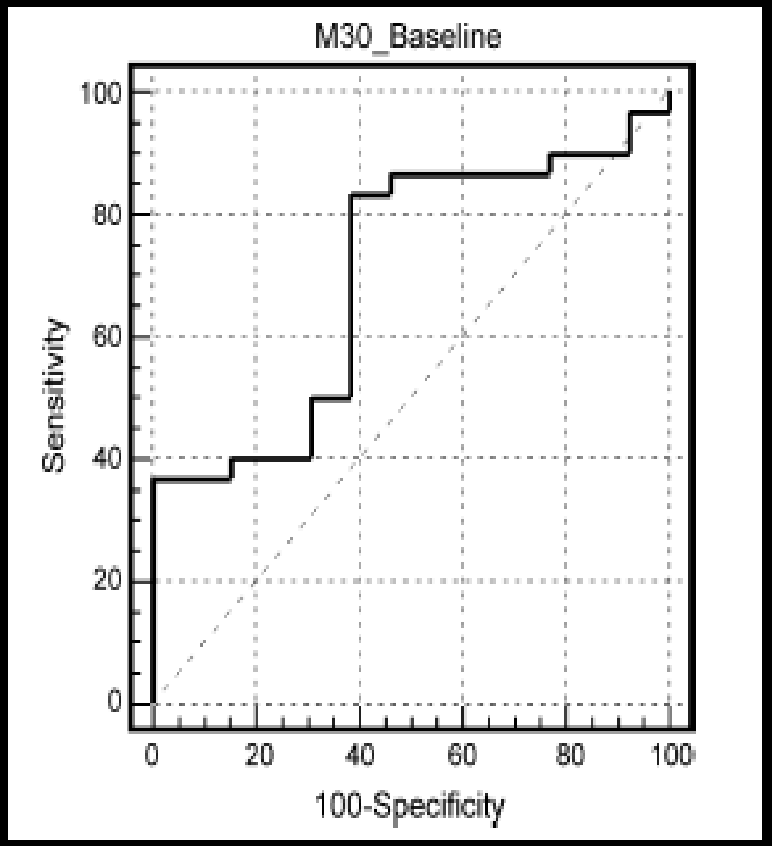
- **Best cut off value= 43.8 U/L (for prediction of death !)**

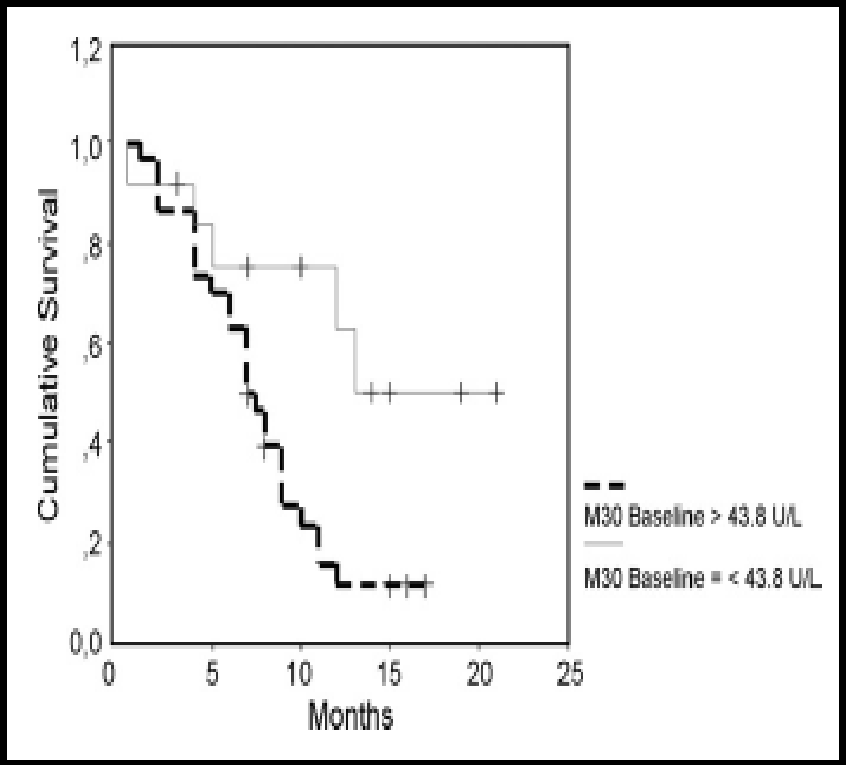
- **AUC=0.700**
(95% CI=0.541-0.830)

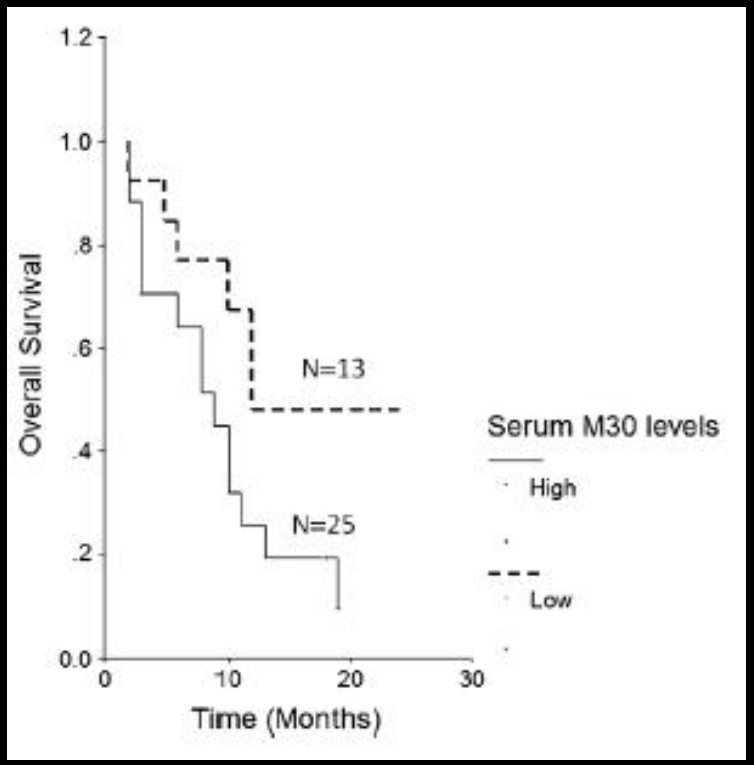
- **Sensitivity=83.3**

- **Specificity=61.5**

- **Positive predictive value=83.3**

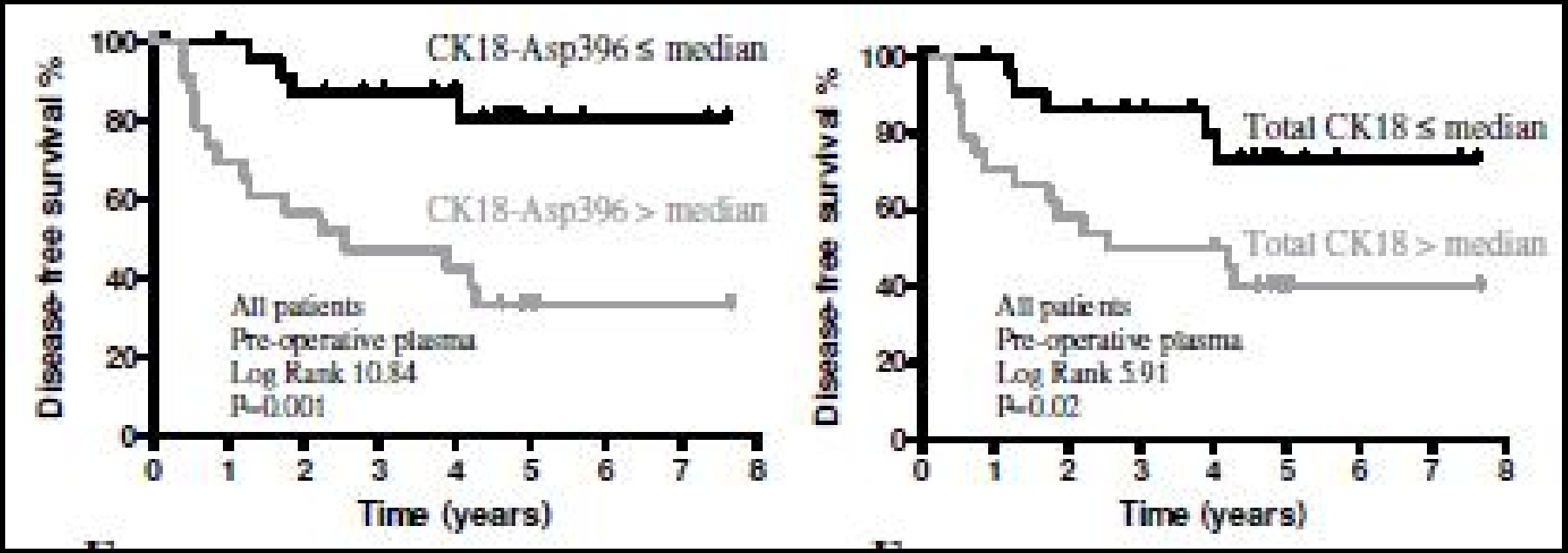




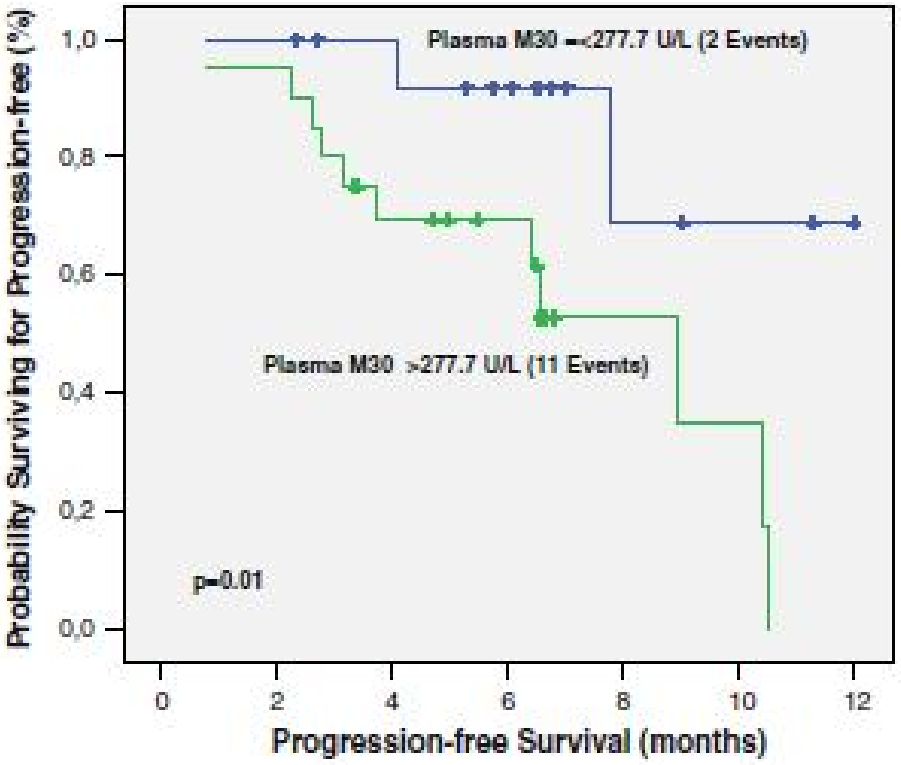


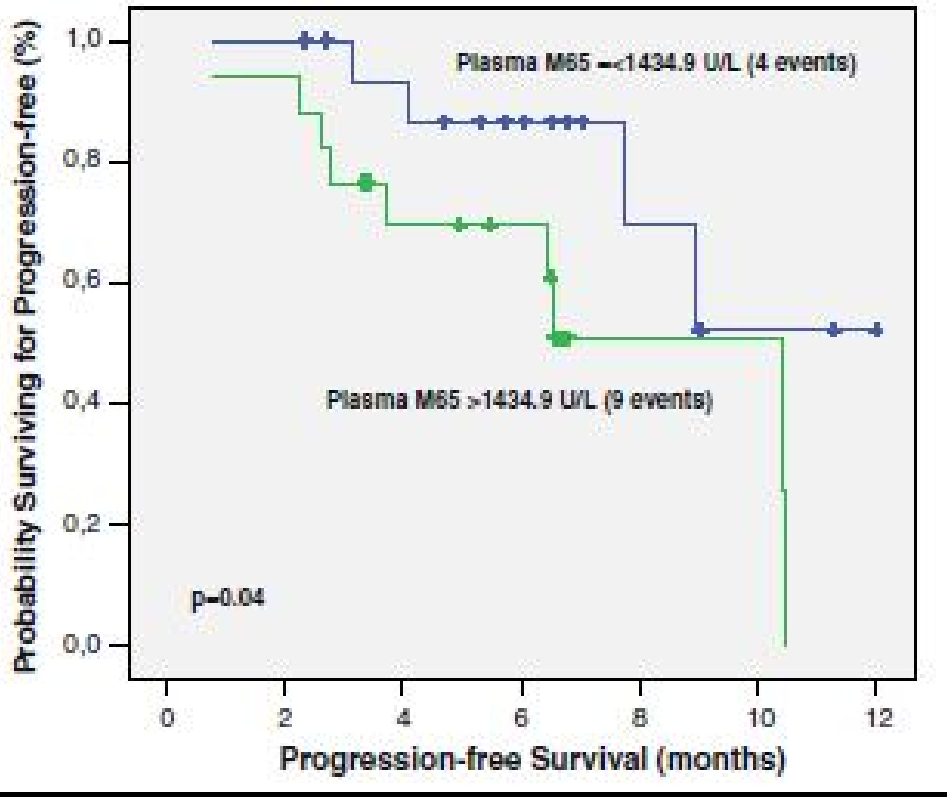
Yaman E, Int Immunopharma, 2010

Gastric Cancer!!



Koelink PJ, BMC Cancer, 2011

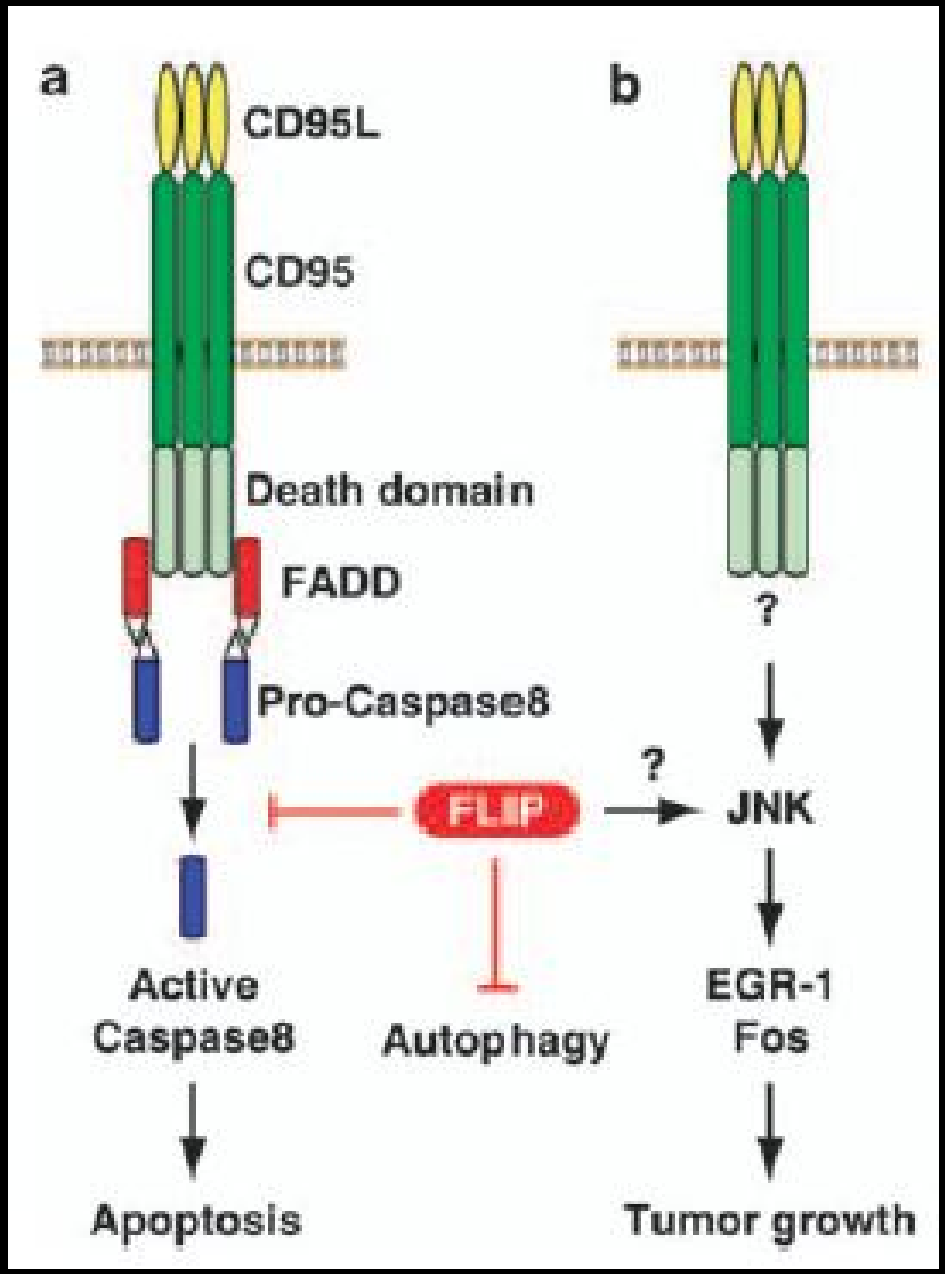




Bilici A, et al., Cancer Chemother Pharmacol, 2011

- Apoptosis promotes early tumorigenesis
  Tang D et al. ONCOGENE , 2011

……. studies suggest that
apoptosis can exert tumor-promoting
as well as tumor-supressing functions,
in a context and cell type spesific manner.


a
CD95L
CD95
Death domain
FADD
Pro-Caspase8
FLIP
Active
Caspase8
Autophagy
Apoptosis
b
?
?
JNK
EGR-1
Fos
Tumor growth

research article

Chemotherapy increases caspase-cleaved cytokeratin 18 in the serum of breast cancer patients

Engin Ulukaya[1], Esra Karaagac[1], Ferda Ari[2], Arzu Y. Oral[1], Saduman B. Adim[3], Asuman H. Tokullugil[1], Türkkan Evrensel[4]

[1] Medical School of Uludag University, Clinical Biochemistry Department, Bursa, Turkey
[2] Science and Art Faculty of Uludag University, Biology Department, Bursa, Turkey
[3] Medical School of Uludag University, Pathology Department, Bursa, Turkey
[4] Medical School of Uludag University, Medical Oncology Department, Bursa, Turkey

Radiol Oncol 2011; 45(2): 116-122. **doi:10.2478/v10019-011-0006-7**

Ulukaya et al., Radiol Oncol 2011

M30-antigen level (U/L)	Before chemotherapy (baseline level)	24 h after chemotherapy	48 h after chemotherapy
Mean (±S.D)	316 (±564)	809 (±1526)	584 (±874)
(min-max)	96-2010	98-4986	82-2586
Median	136	143	150
p-Value		<0.05*	>0.05**

Apoptozis parametreleri kanser hastalarında kemoterapiye yanıtı ve prognozu belirlemede yardımcı olabilirler

Holdenrieder and Stieber, Cancer Biomarkers, 2010

M30 mi yoksa M65 mi ya da HER İKİSİ…?

Apoptosis or Necrosis
Or BOTH…?

M30 or M65, or BOTH ???

Table 4. Increased levels of CK18 in patient serum during treatment using different agents

Treatment	Increased levels of CK18 during therapy (%)	
	Caspase cleaved*	Total*
Docetaxel (breast)†	19.8 ($P = 0.0089$)	16.5 (NS)
Docetaxel (prostate)‡	18.7 ($P < 0.0001$)	21.4 ($P < 0.0002$)
Vinorelbine (prostate)‡	7.2 ($P < 0.001$)	6.7 ($P < 0.011$)
Estramustine (prostate)‡	-1 (NS)	8.2 ($P < 0.0001$)
CEF (breast)§	12.9 ($P < 0.00001$)	32.7 ($P < 0.00001$)

*Increased median levels of CK18-Asp^{396} and total CK18 (measured by the M30-Apoptosense and M65 ELISA assays).
† Increase over pretherapy levels at 72 h.
‡ Increase over pretherapy levels 48 h (prostate data are from ref. 13).
§ Increase over pretherapy levels at 24 h.

- M30-based basal apoptotic rate assays reflect apoptosis accurately and are more amenable to clinical application than existing apoptosis assays.

Zhang, Clin Cancer Res, 2010

KARDİYOVASKÜLER HASTALIKLAR ve APOPTOZİS

Serial changes in circulating M30 antigen, a biomarker of apoptosis, in patients with acute coronary syndromes: relationship with the severity of coronary artery disease

Tunay Senturk[a], Ali Aydinlar[a], Yusuf Yilmaz[b], Arzu Yilmaztepe Oral[c], Osman Ozdabakoglu[a] and Engin Ulukaya[c]

Conclusion Serum levels of the apoptotic marker M30 peak at 24 h after AMI and reflects the extent of coronary artery disease in this patient group. ***Coron Artery Dis*** 20:494–498

NASH ve Apoptozis

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 February 14; 13(6): 837-844
World Journal of Gastroenterology ISSN 1007-9327

VIRAL HEPATITIS

Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis

Yusuf Yilmaz, Enver Dolar, Engin Ulukaya, Semra Akgoz, Murat Keskin, Murat Kiyici, Sibel Aker, Arzu Yilmaztepe, Selim Gurel, Macit Gulten, Selim Giray Nak

Table 4 Concentrations of serum M30-antigen and M65-antigen in the study groups

Biomarker	Definitive NASH (n = 45)	Borderline NASH (n = 24)	Simple steatosis (n = 9)	Normal tissue (n = 5)	Healthy volunteers (n = 49)	No-NASH (n = 38)	P^1
M30-antigen (IU/L)	200.4 ± 183.1	69.8 ± 37.1	60.1 ± 36.8	81.0 ± 17.5	43.3 ± 45.9	69.0 ± 34.9	< 0.001
P^2	Reference category	< 0.001	< 0.01	NS	< 0.001	< 0.001	
M65-antigen (IU/L)	362.8 ± 178.7	210.6 ± 59.6	215.3 ± 78.2	207.0 ± 47.4	150.9 ± 44.1	211.2 ± 61.5	< 0.001
P^2	Reference category	< 0.001	< 0.01	< 0.05	< 0.001	< 0.001	

[1]*P* value calculated using Kruskal-Wallis test. [2]Comparisons between definitive NASH patients and other patient groups were performed by Mann-Whitney *U* Test.

In summary, the results of the present study suggest that noninvasive monitoring of different forms of CK18 (M30-antigen and M65-antigen) in sera of patients with suspected NAFLD may represent a reliable tool to differentiate definitive NASH from simple fatty liver.

Journal of Pediatric Gastroenterology and Nutrition
47:481–485 © 2008 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and
North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Cytokeratin 18, a Marker of Cell Death, Is Increased in Children With Suspected Nonalcoholic Fatty Liver Disease

*Miriam B. Vos, †‡Shirish Barve, †Swati Joshi-Barve, §John D. Carew, ¶Peter F. Whitington, and †‡||Craig J. McClain

Current Medicinal Chemistry, 2011, *18*, 725-732

Toward a Biochemical Diagnosis of NASH: Insights From Pathophysiology For Distinguishing Simple Steatosis From Steatohepatitis

Y. Yilmaz*,[1] and E. Ulukaya[2]

[1]*Department of Gastroenterology, Marmara University School of Medicine, 34662 Altunizade, Istanbul, Turkey*

[2]*Department of Biochemistry, Uludag University Medical School, 16059, Bursa, Turkey*

İskemi / Reperfüzyon Hasarı ve Apoptozis

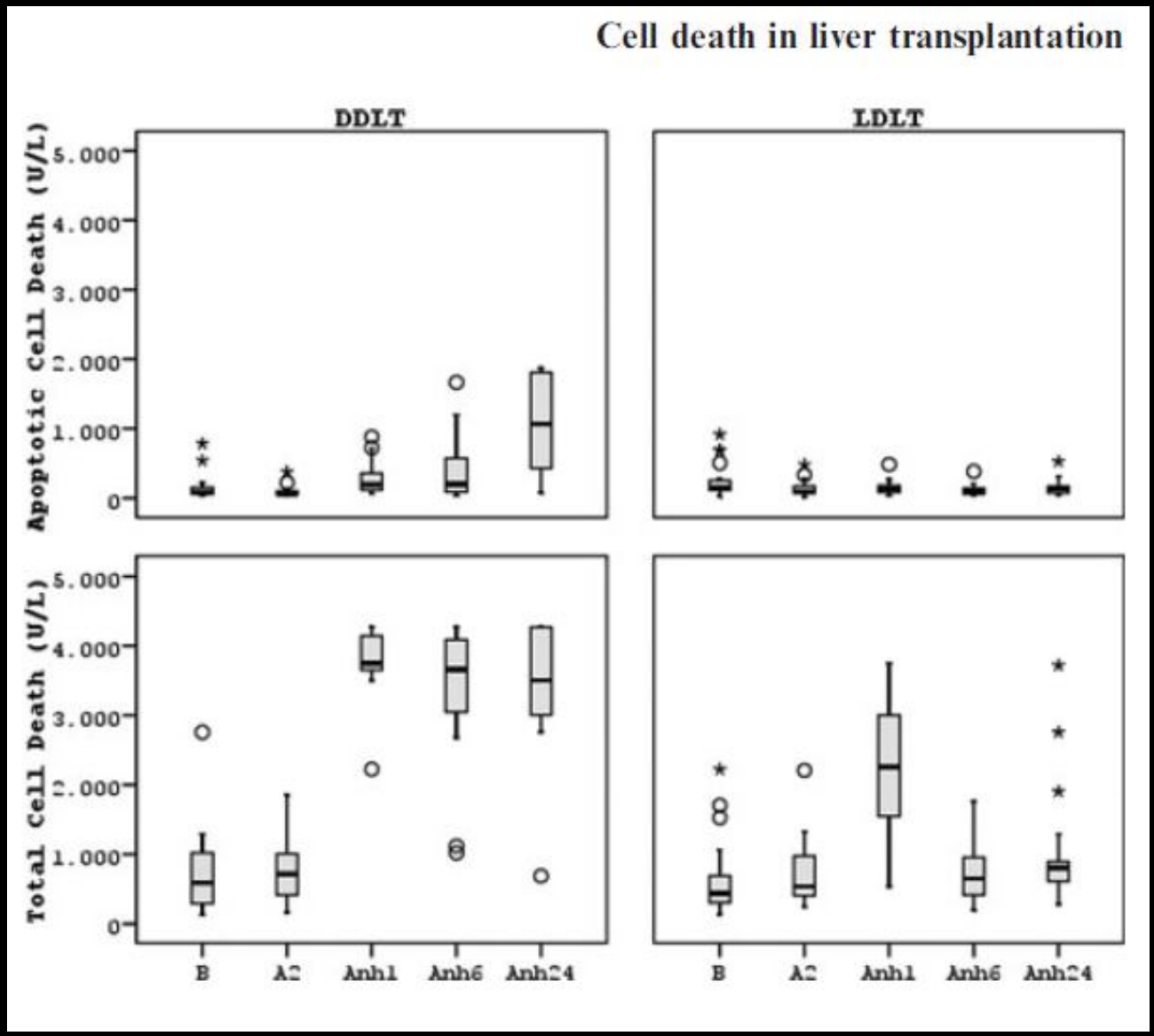
Clin Transplant 2009 DOI: 10.1111/j.1399-0012.2009.01177.x

Clinical Transplantation

Soluble cytokeratin 18 biomarkers may provide information on the type of cell death during early ischemia and reperfusion periods of liver transplantation

Ulukaya S, Ulukaya E, Alper I, Yilmaztepe-Oral A, Kilic M. Soluble cytokeratin 18 biomarkers may provide information of the type of cell death during early ischemia and reperfusion periods of liver transplantation. Clin Transplant 2009 DOI: 10.1111/j.1399-0012.2009.01177.x

Sezgin Ulukaya[a], Engin Ulukaya[b], Isik Alper[a], Arzu Yilmaztepe-Oral[b] and Murat Kilic[c]


Cell death in liver transplantation
DDLT
LDLT
Apoptotic Cell Death (U/L)
Total Cell Death (U/L)
5.000
4.000
3.000
2.000
1.000
0
B
A2
Anh1
Anh6
Anh24

EXPERT REVIEWS

Utilization of cytokeratin-based biomarkers for pharmacodynamic studies

Expert Rev. Mol. Diagn. 10(3), 353–359 (2010)

Stig Linder†, Maria Hägg Olofsson, Richard Herrmann and Engin Ulukaya

Cytokeratin (CK)18 is a useful serum biomarker for the determination of cell death of epithelial-derived tumors (carcinomas). ELISAs are available for caspase-cleaved CK18 (M30) released from apoptotic cells, or total CK18 (M65) released by cells undergoing cell death by any cause. These assays have been demonstrated to have prognostic or predictive utility in various types of carcinomas. Encouraging data have been reported by different investigators with regard to the

ÖZET

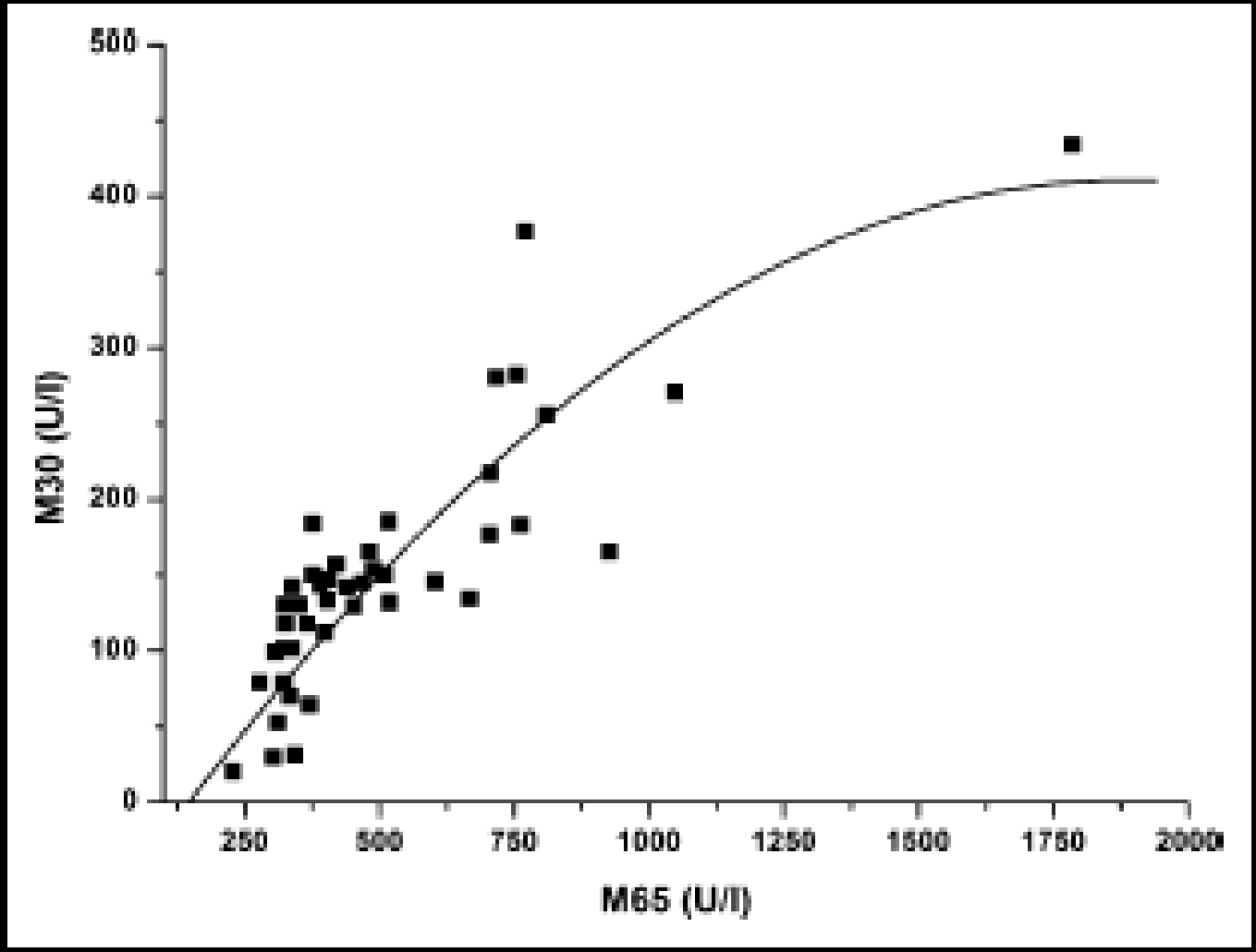
- Hücre ölümü en az hücre yaşamı kadar önemlidir ve birçok hastalıkla veya biyolojik durumla doğrudan ilişkilidir.
- Ölüm ve yaşam arasındaki denge bozulmamalıdır
- Serum M30 (ve/veya M65) ölçümleri kanser hastalarında kemoterapinin etkinliğini izlemede, NASH hastalarında ise ayırıcı tanıda ümit vaat eden yeni biobelirteçlerdir.

Teşekkürler...

Arzu Yılmaztepe Oral
Yusuf Yılmaz
Meryem Yılmaz
Mehmet Karadağ
Mutlu Demiray

Stig Linder (Karolinska Institute, SWEDEN)

Ausch C, J Gastrointest Surg, 2009



Fas expression is associated with a better prognosis in laryngeal squamous cell carcinoma

Asensio, 2007

Tümör Hücrelerinin Apoptozisden Kurtulma Mekanizmaları

- **Fas ve Fas Ligand (Fas-L) interaksiyonları**
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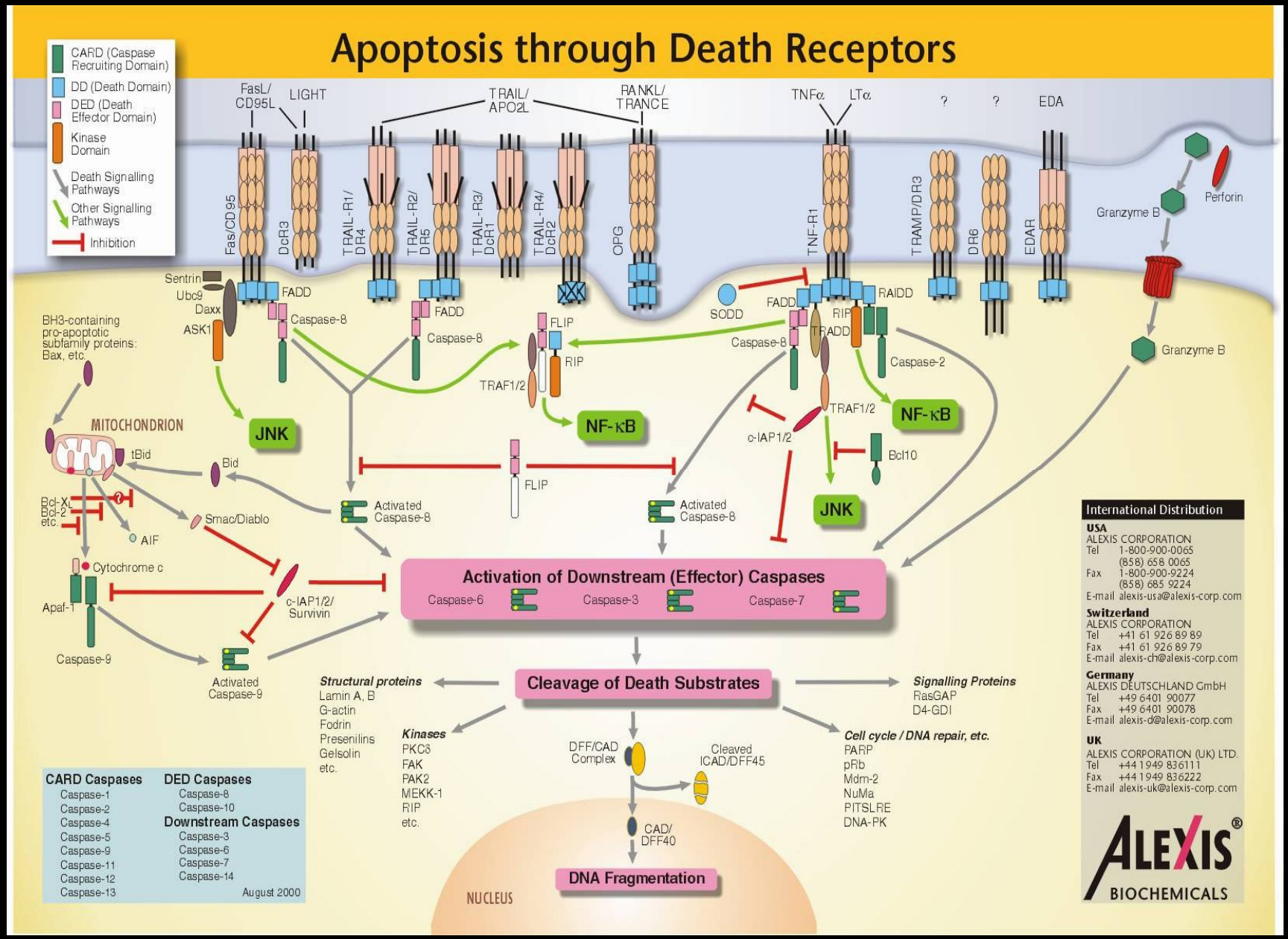
Apoptosis through Death Receptors
CARD (Caspase Recruiting Domain)
DD (Death Domain)
DED (Death Effector Domain)
Kinase Domain
Death Signalling Pathways
Other Signalling Pathways
Inhibition
FasL/ CD95L
LIGHT
TRAIL/ APO2L
RANKL/ TRANCE
TNFα
LTα
?
?
EDA
Fas/CD95
DcR3
TRAIL-R1/ DR4
TRAIL-R2/ DR5
TRAIL-R3/ DcR1
TRAIL-R4/ DcR2
OPG
TNF-R1
TRAMP/DR3
DR6
EDAR
Perforin
Granzyme B
Granzyme B
Sentrin
Ubc9
Daxx
ASK1
FADD
Caspase-8
FADD
Caspase-8
FLIP
RIP
TRAF1/2
NF-κB
SODD
FADD
RIP
TRADD
RAIDD
Caspase-8
Caspase-2
TRAF1/2
NF-κB
c-IAP1/2
Bcl10
BH3-containing pro-apoptotic subfamily proteins: Bax, etc.
MITOCHONDRION
tBid
Bid
JNK
FLIP
Activated Caspase-8
Activated Caspase-8
JNK
Bcl-Xl
Bcl-2
etc.
Smac/Diablo
AIF
Cytochrome c
Apaf-1
c-IAP1/2/ Survivin
Caspase-9
Activated Caspase-9
Activation of Downstream (Effector) Caspases
Caspase-6
Caspase-3
Caspase-7
Cleavage of Death Substrates
Structural proteins
Lamin A, B
G-actin
Fodrin
Presenilins
Gelsolin
etc.
Kinases
PKCδ
FAK
PAK2
MEKK-1
RIP
etc.
DFF/CAD Complex
Cleaved ICAD/DFF45
CAD/ DFF40
DNA Fragmentation
NUCLEUS
Signalling Proteins
RasGAP
D4-GDI
Cell cycle / DNA repair, etc.
PARP
pRb
Mdm-2
NuMa
PITSLRE
DNA-PK
CARD Caspases
Caspase-1
Caspase-2
Caspase-4
Caspase-5
Caspase-9
Caspase-11
Caspase-12
Caspase-13
DED Caspases
Caspase-8
Caspase-10
Downstream Caspases
Caspase-3
Caspase-6
Caspase-7
Caspase-14
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BIOCHEMICALS

TRAIL-R4 decoy receptor expression is correlated with decreased survival in patients with prostate carcinoma

Köksal, 2008

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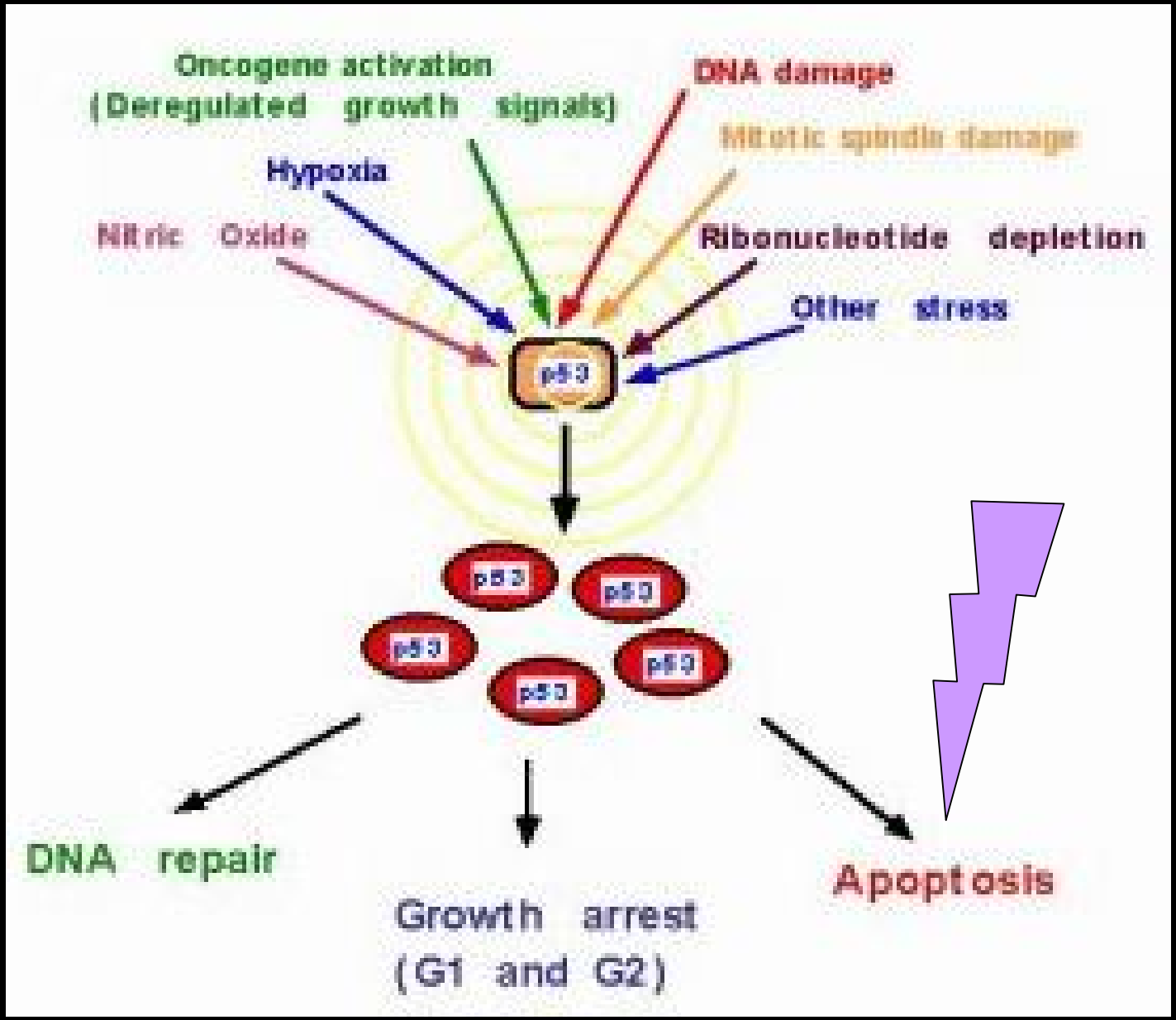
Oncogene activation
(Deregulated growth signals)
DNA damage
Mitotic spindle damage
Hypoxia
Nitric Oxide
Ribonucleotide depletion
Other stress
p53
p53
p53
p53
p53
p53
DNA repair
Growth arrest
(G1 and G2)
Apoptosis

The routine evaluation of p53 and Ki67 levels could be a useful tool in identification of patient with more aggressive disease and contribute to a better therapeutic approach. GASTRIC CANCER!!!

Tzanakis, 2009

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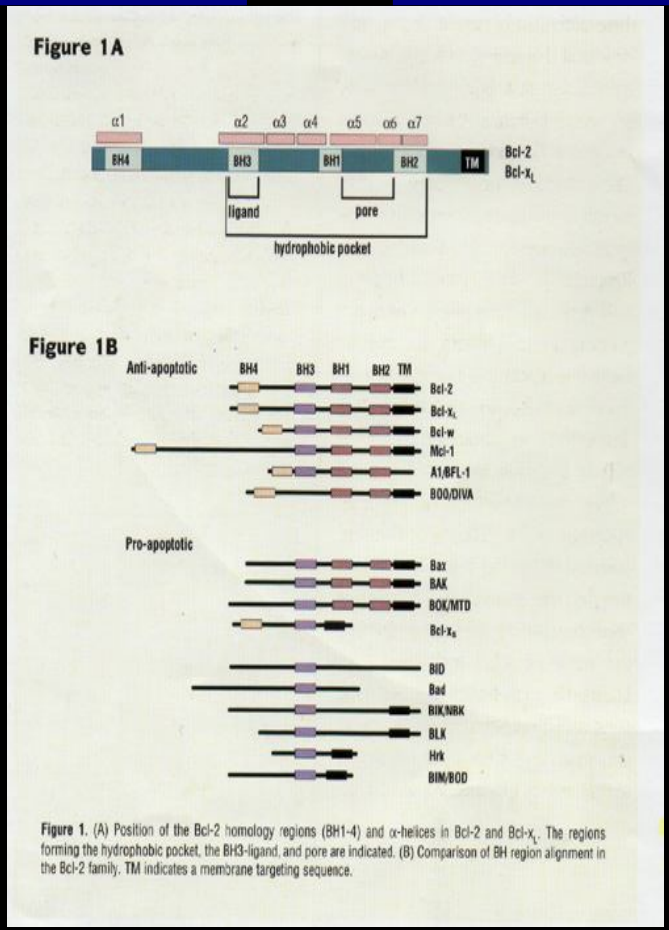
Bcl-2 AİLESİ

Anti-apoptotik

Pro-apoptotik



Bax mutasyonla inaktive olursa kemoterapötik ajanlarla indüklenen apoptozise karşı direnç gelişir

LeBlanc, et al., 2002

Poor outcome in patients with diffuse large B-cell lymphoma is associated with high percentage of bcl-2 and Ki 67-positive tumor cells.

Jovanović, 2009

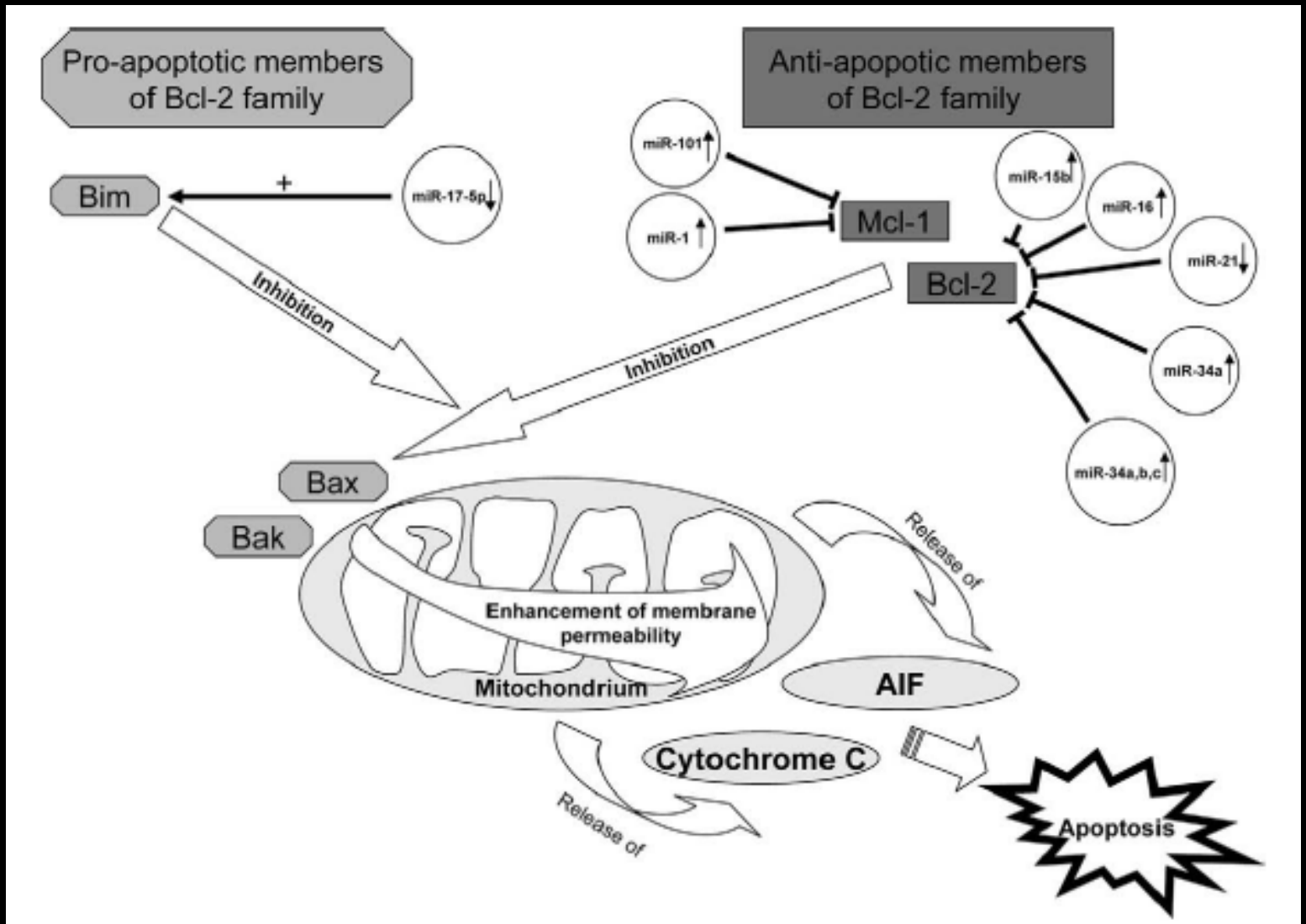


Fig. 1 – Regulation of Bcl-2 pathway by different miRNAs.

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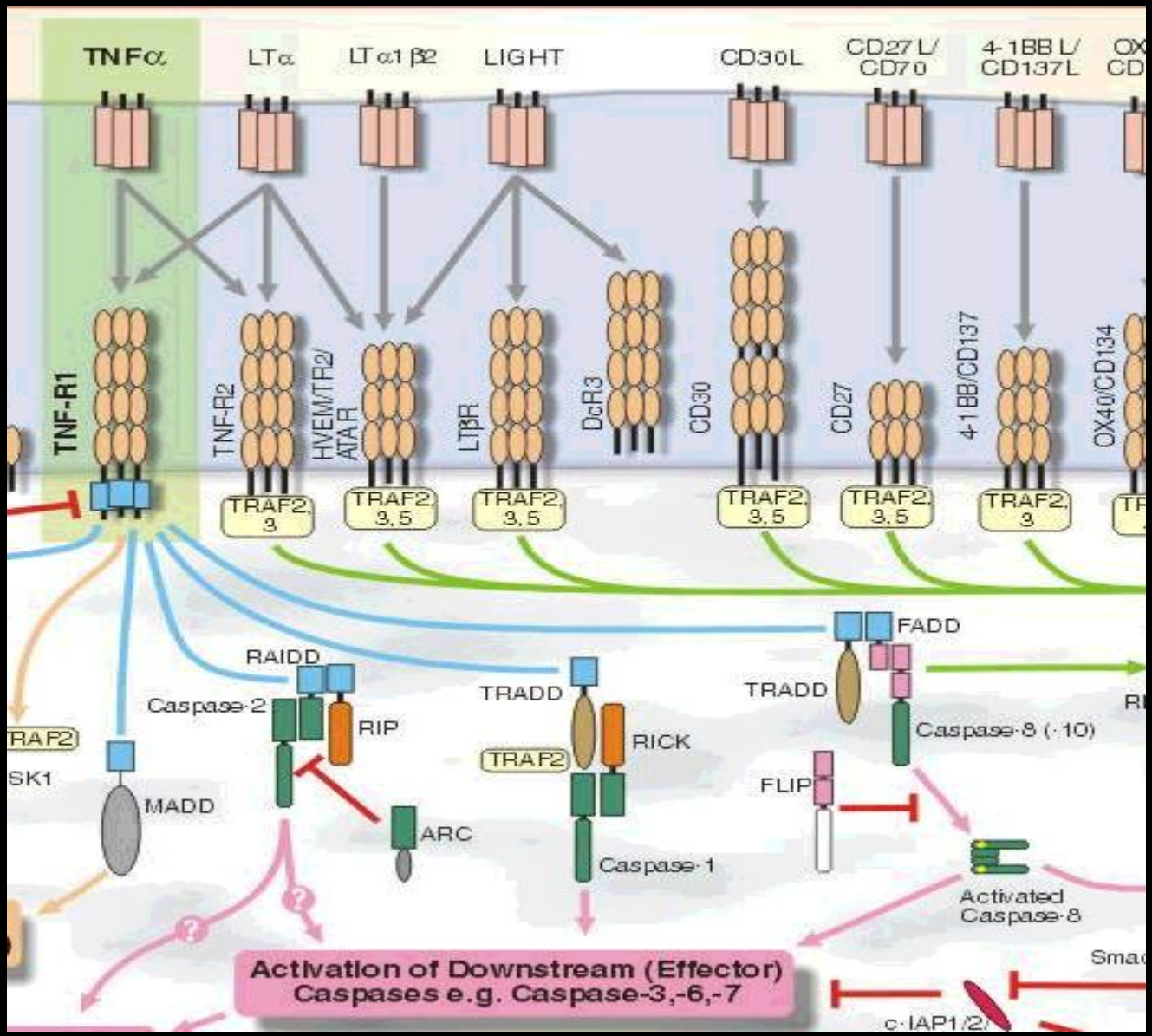
TNFα
LTα
LTα1β2
LIGHT
CD30L
CD27 L/
CD70
4-1BB L/
CD137L
TNF-R1
TNF-R2
HVEM/TR2/
ATAR
LTβR
DcR3
CD30
CD27
4-1BB/CD137
OX40/CD134
TRAF2,
3
TRAF2,
3,5
TRAF2,
3,5
TRAF2,
3,5
TRAF2,
3,5
TRAF2,
3
FADD
TRADD
Caspase-8 (-10)
RAIDD
Caspase-2
RIP
ARC
TRADD
RICK
TRAF2
Caspase-1
FLIP
Activated
Caspase-8
TRAF2
SK1
MADD
Activation of Downstream (Effector)
Caspases e.g. Caspase-3,-6,-7
c-IAP1/2

Overexpression of FLIP is an independent marker of poor prognosis in colorectal cancer patients

Ullenhag , 2007

Tümör Hücrelerinin Apoptozisden Kurtulma Mekanizmaları

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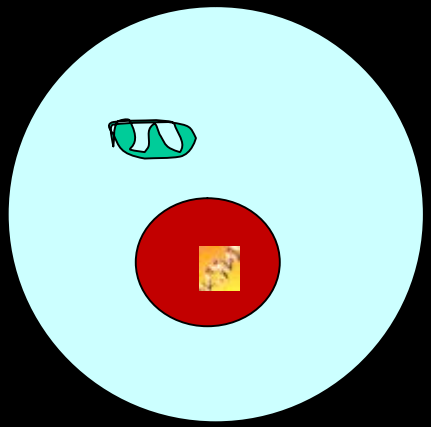
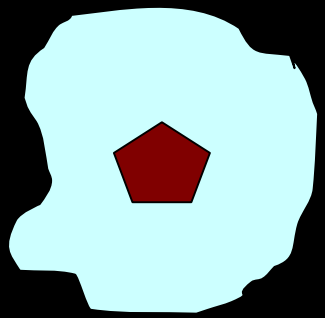
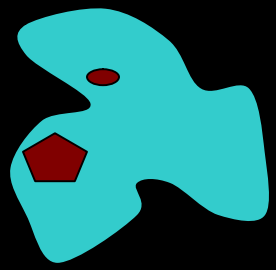
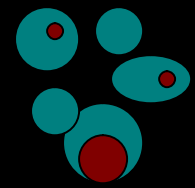
THE CELL UNDERGOING APOPTOSIS

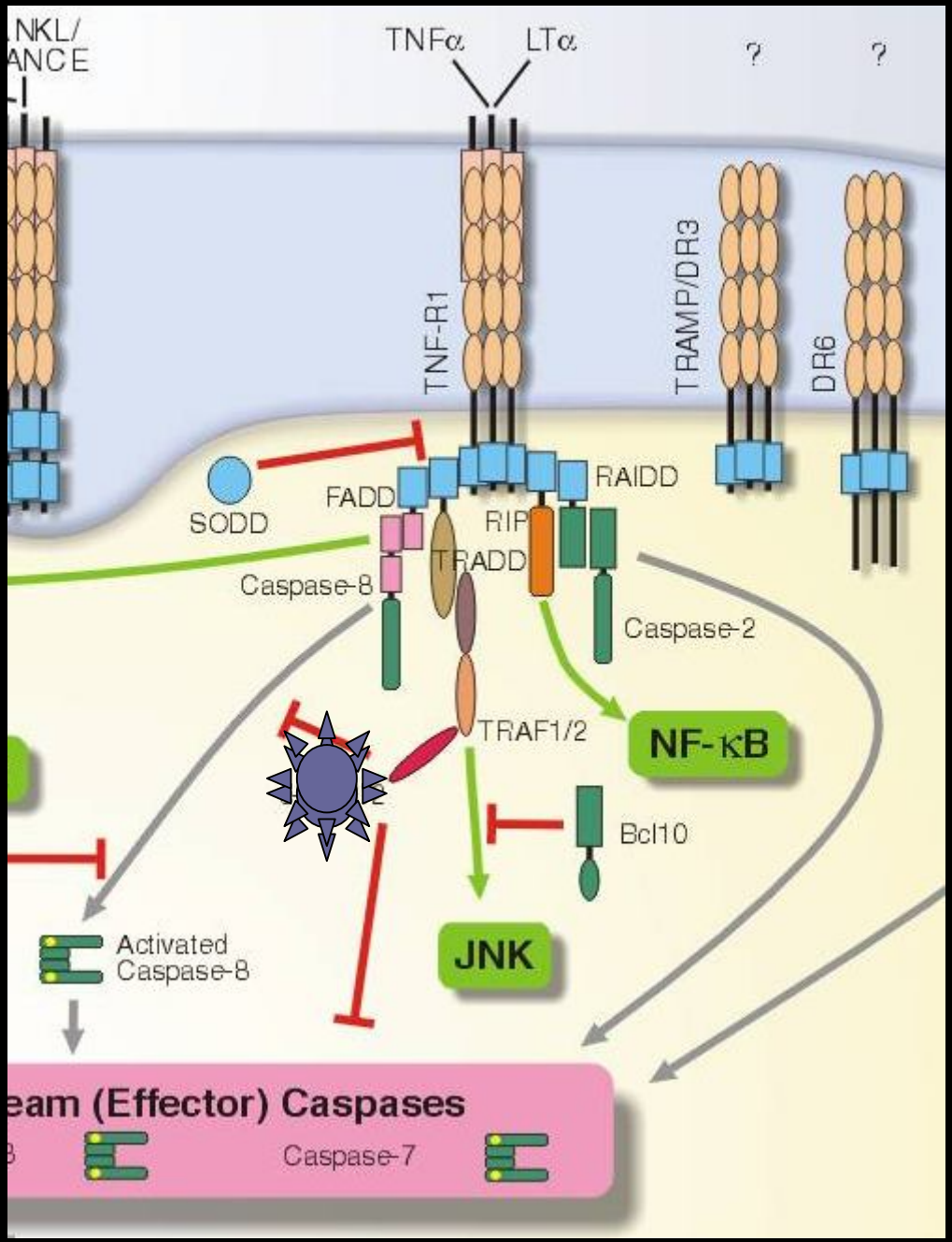
Triggers	Modulators	Effectors	Substrates	DEATH
1. Occupation of death receptors . Fas . TNFR1 . DR3, 4, 5				
2. Granzymes	. FADD . TRADD . FLIP . Bcl-2 family . Cytochrome c . p53 . Mdm2	. Caspases	. Many cellular proteins . DNA	. Apoptotic body formation . Phagocytosis
3. Growth factor depletion				
4. Genomic damage . Radiation . Chemotherapy				

Dr. E Ulukaya

Kaspazların İnhibisyonu

A. Virüsler

B. Sellüler inhibitörler


NKL/
ANCE
TNFα
LTα
?
?
TNF-R1
TRAMP/DR3
DR6
SODD
FADD
RAIDD
RIP
TRADD
Caspase-8
Caspase-2
TRAF1/2
NF-κB
Bcl10
Activated
Caspase-8
JNK
eam (Effector) Caspases
Caspase-7

Bcl-Xl ve cIAP1, karaciğer kanserinin gelişmesinde rol oynarlar

Fabregat, 2007

mesaj…

- M30 antijen yeni bir apoptozis biobelirteci olarak kemoterapiye yanıtı tahmin etmede faydalı olabilir. Fakat, rutin kullanım için henüz erkendir.