Buněčný cyklus a apoptóza v karcinogenezi

Význam regulace buněčného cyklu
Úloha apoptózy v karcinogenezi
Oxidativní stres
Oxidativní metabolismus mastných kyselin

Význam regulace buněčného cyklu

Buněčný cyklus a jeho regulace

Pokud buňka obdrží nezbytné informace nebo mimobuněčný podnět (hormony, cytokiny, kontakt se sousední buňkou nebo substrátem) začne se dělit. Jinak zůstává v klidovém stavu - ***G0***.

Kontrolní body G1-S a G2-M

Regulační molekuly

Proteinové kinázy - cyklin (regulační jednotka) + cyklin dependentní kinázy (katalytická subjednotka):

- Cyklin D + Cdk4 a Cdk6 - přechod ***G1-S***
- Cyklin E + Cdk2 - začátek ***S fáze***
- Cyklin A + Cdk2 - ***S*** a ***G2***
- CyklinB + Cdk1 - přechod ***G2-M***

Aktivita komplexů je regulována fosforylací treoninových a tyrosinových residuí

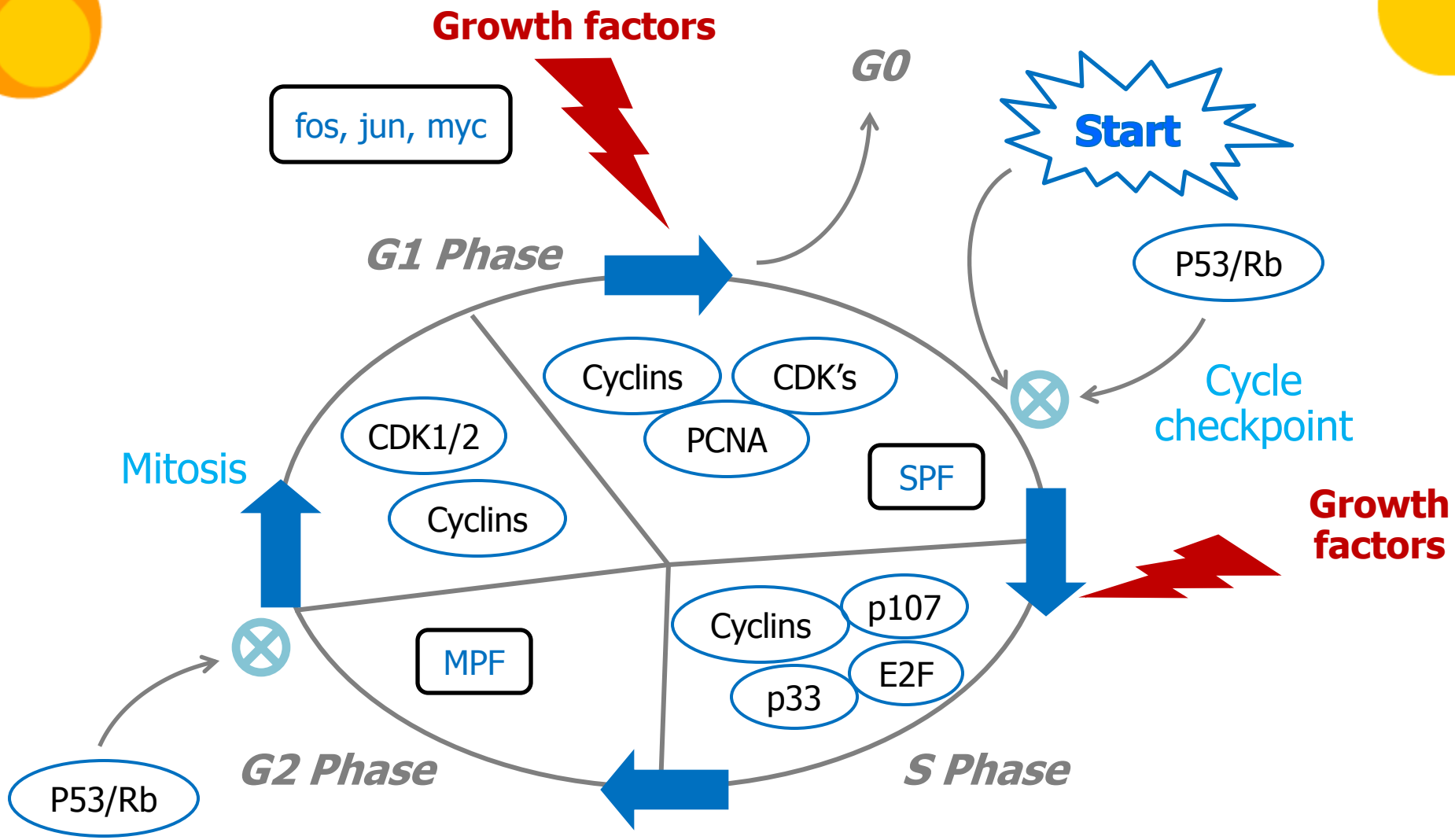
Komplexy inaktivovány degradací cyklinů

Inhibitory Cdk - 2 hlavní rodiny: p21, p27, p57 (širší specifita) a p15, p16, p18, p19 - váží se přímp na Cdk2 a Cdk4 (specif pro cyklin D závislé kinázy)

Několik stupňů kontroly, funkce různých komponent jsou vzájemně propojeny - cíl pro mutace i epigenetické změny

Cyklin E - aberantně exprimován u řady nádorů prsu

Funkce cyklinu A ovlivněny spojením s proteiny kodujícími DNA viry (papilloma virus - nádory děložního čípku)

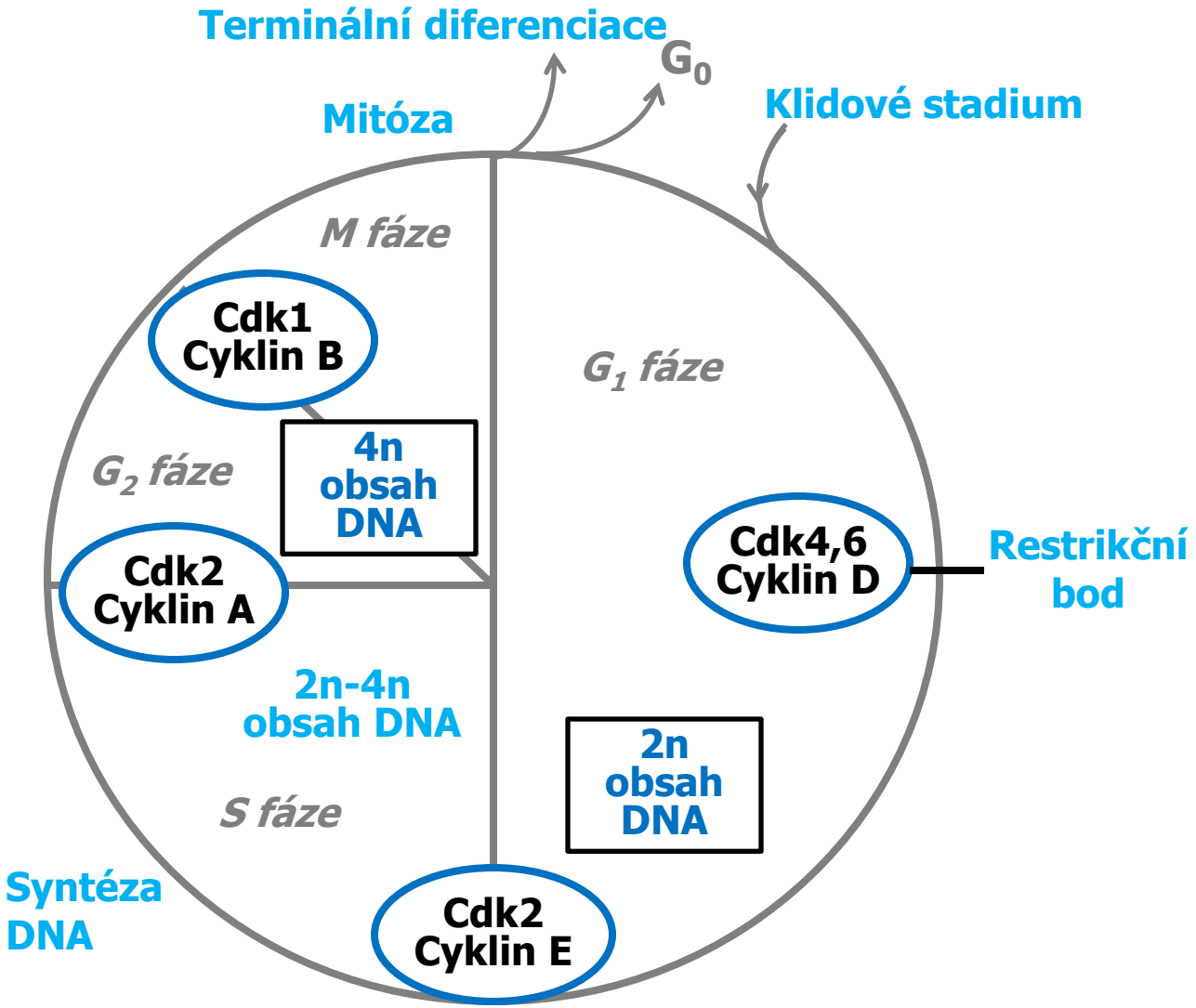


Buněčný cyklus s kontrolními faktory a hlavními kontrolními body pro přechod z jedné fáze do druhé.

- p107 = protein ve vztahu k Rb;
- p33 = cyklin-dependentní protein kináza;
- p53/Rb = inhibiční proteiny;
- CDK = cyklin-dependentní kináza;
- PCNA = proliferating cell nuclear antigen.
- MPF = M-phase promoting factor (p34 a cyklin B);
- SPF = S-phase promoting factor;
- START = rozhodující bod pro dělení nebo diferenciaci;
- E2F = transkripční faktor v syntéze DNA;

Schéma progrese normálního buněčného cyklu.

Po ukončení mitózy může buňka terminálně diferencovat, vstoupit do klidového stadia nebo znovu vstoupit do buněčného cyklu. Progrese buněčným cyklem je regulována různými komplexy cdk-cyklin.



Retinoblastoma protein pRB

Produkt "retinoblastoma susceptibility" genu Rb-1 - první klonovaný nádorově supresorový gen - homozygotní mutace - retinoblastom
RB protein je substrátem Cdks a jeho funkce je inhibována fosforylací (na serinech a treoninech) nebo virovými onkoproteiny.

Fosforylací se uvolňuje transkripční faktor E2F, který reguluje transkripci genů kritických pro syntézu DNA a S-fázi.

Fosforylace vykazuje pravidelnou oscilaci v průběhu bun. cyklu. U nesynchronizovaných buněk - half-life RB asi 30 min.

Rychlý obrat naznačuje, že RB je reverzibilně fosforylován pomocí kináz a fosfatáz. Během G1 fáze je RB fosforylován - progrese bun. cyklu - důležitost komplexu cdk2-cyklin E a cdk2a 4 a cyklin D

Antimitogeny a induktory diferenciace vedou k defosforylaci a podporují tak zástavu cyklu u proliferujících buněk.

"Electromobility shift assay" - fosforylovaný RB migruje jako samostatný band u klidových buněk a jako soubor 4-5 bandů u proliferujících buněk.

Fosforylace zpomaluje elfo mobilitu - horní bandy odpovídají fosforylovanému RB.

Protein p53

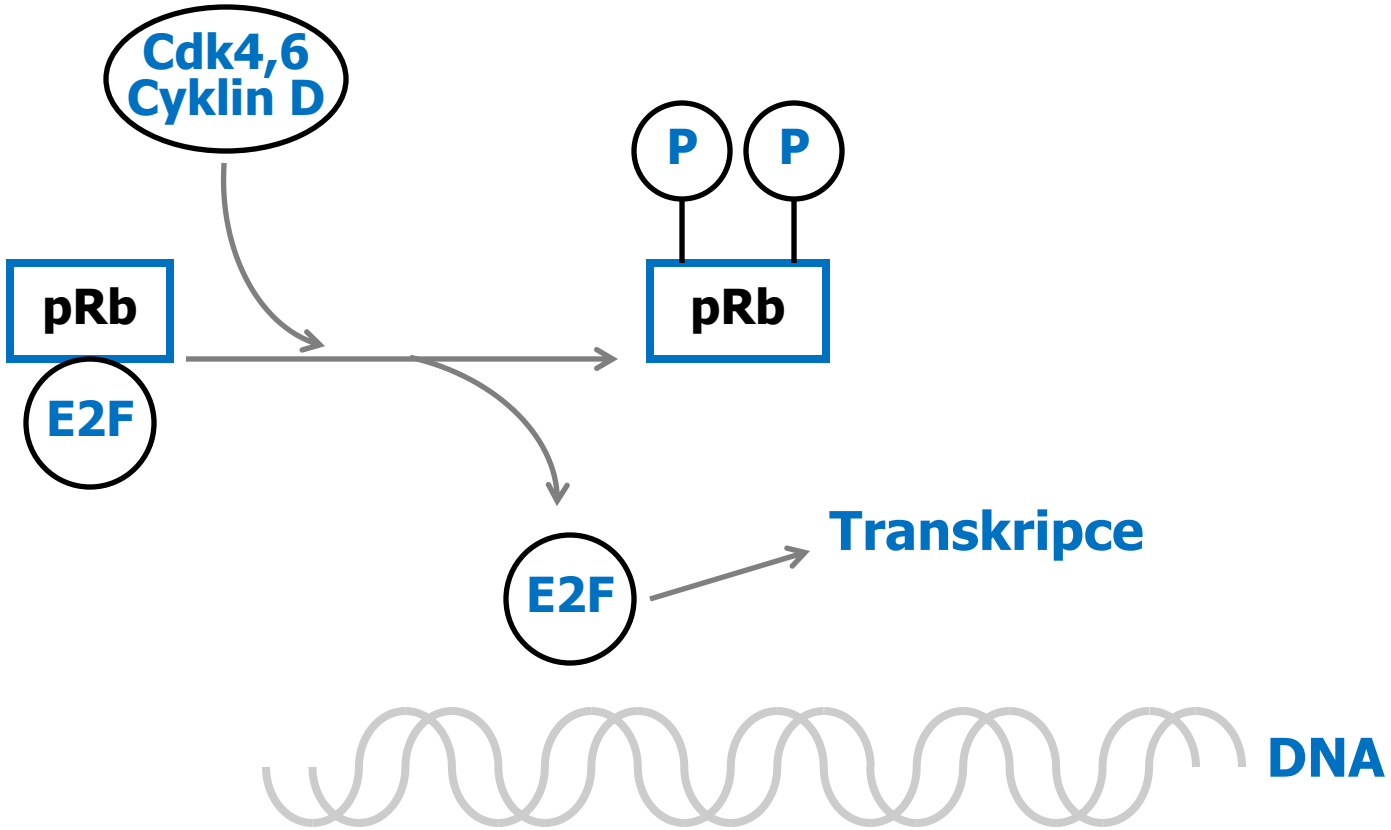
úloha v proliferaci a apoptóze

aktivován poškozením DNA (záření, chem. látky, cytostatika) - aktivace vyúsťuje v apoptózu (přes bax) nebo zástavu bun. cyklu (p21) - čas pro reparaci u nádorů často deficientní nebo mutován - regulace E2F, p19

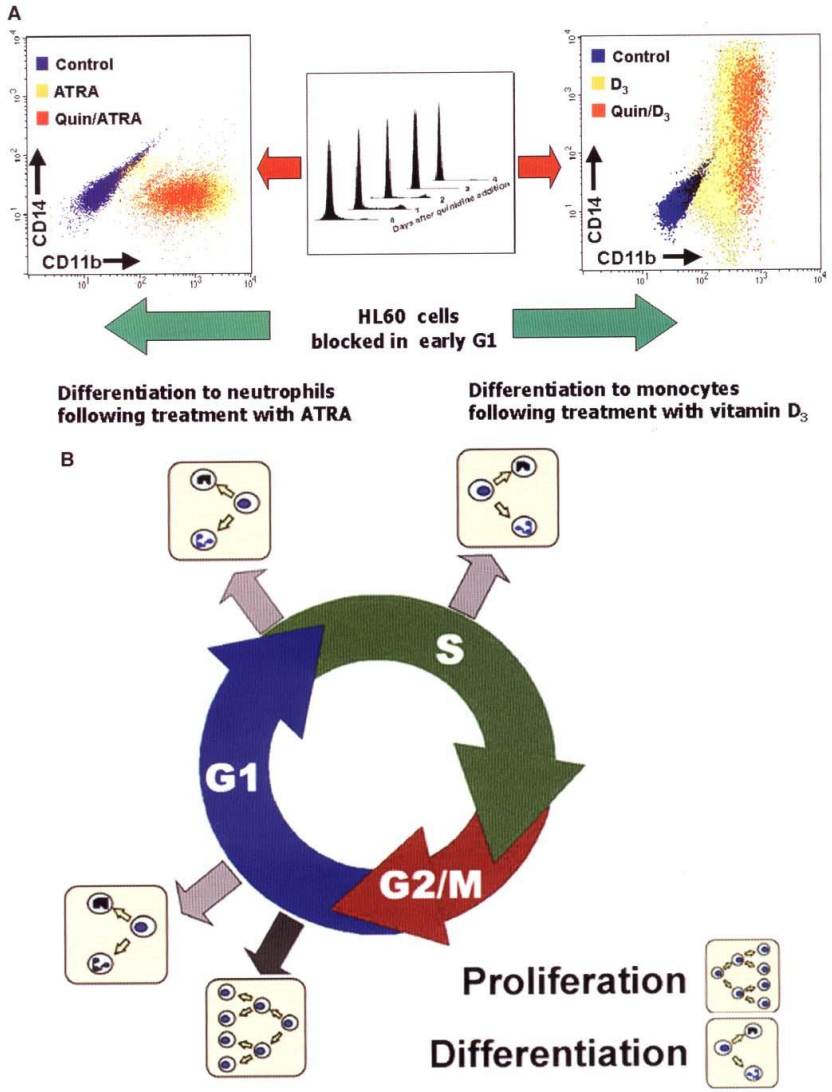
Fosforylace RB je ovlivňována řadou biologicky aktivních molekul jako jsou mitogeny, antimitogeny a induktory diferenciace. Mitogeny stimulují fosforylaci a klidové buňky přecházejí do cyklu.

Schéma aktivace E2F komplexem cdk-cyklin.

Cdk4 nebo cdk6 v komplexu s cyklinem D fosforylují pRb, který uvolňuje E2F pro transkripci genů nutných k progresi buněčného cyklu.



Vztah mezi buněčným cyklem a diferenciací



Zástava buněčného cyklu indukovaná poškozením DNA

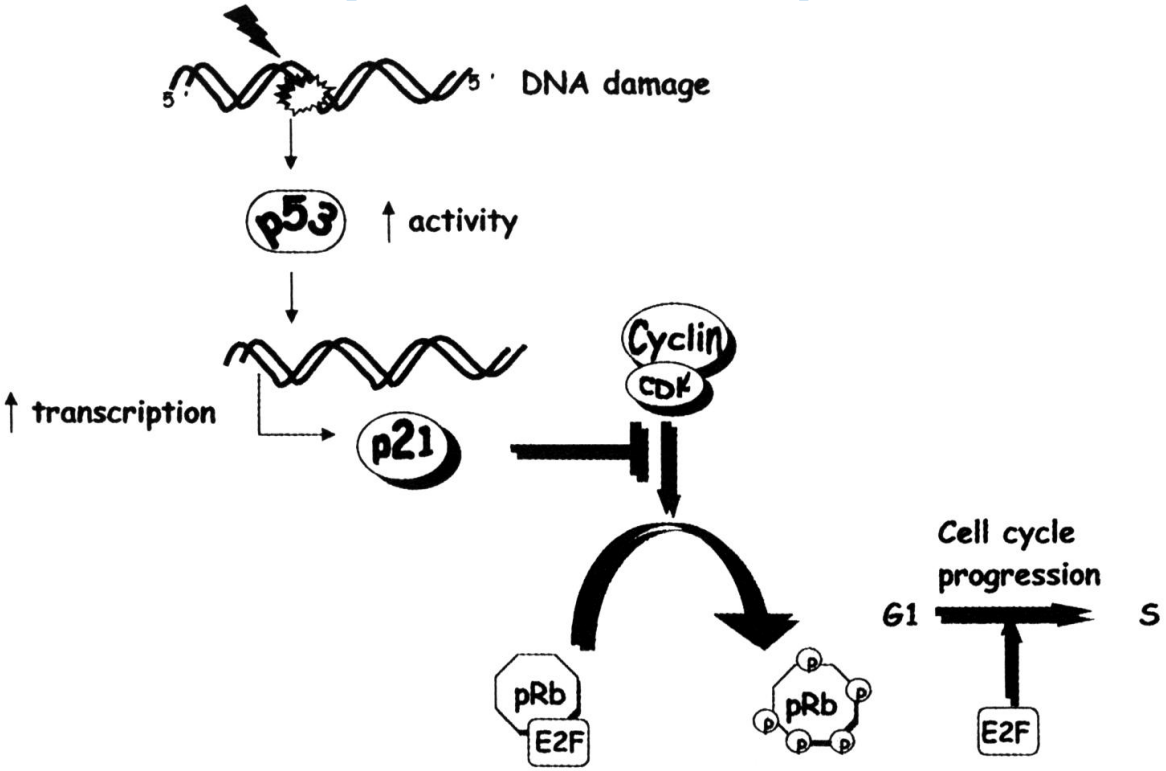


Figure 2. DNA damage-induced cell-cycle arrest in G-1 phase by p21. Increase in p53 activity in response to DNA damage leads to transcriptional activation of several genes including p21. Upregulation of p21 inhibits cdk2-cyclin kinase activity. Phosphorylation of pRb in late G-1 by cdk-cyclin kinases is necessary to release E2F transcription factors which then activate several genes that are important for G-1 to S phase transition. p21 prevents phosphorylation of pRb and causes G-1 arrest. DNA damage-induced G-1 arrest is defective in cells from p21 null mice.

Při poškození DNA vzrůstá aktivita p53 – transkripční aktivace p21 – inhibice aktivity cdk2 cyklin kinázy. Fosforylace Rb v pozdní G1 fázi cdk je nezbytná pro uvolnění tr. Faktoru E2F aktivujícího řadu genů nutných pro přechod G1-S. p21 zabraňuje fosforylaci Rb a indukuje zástavu v G1 fázi.

Mutace a nefunkčnost těchto molekul – deregulace bun. cyklu – podpora rozvoje nádorů

Regulace přechodu G1/S fáze

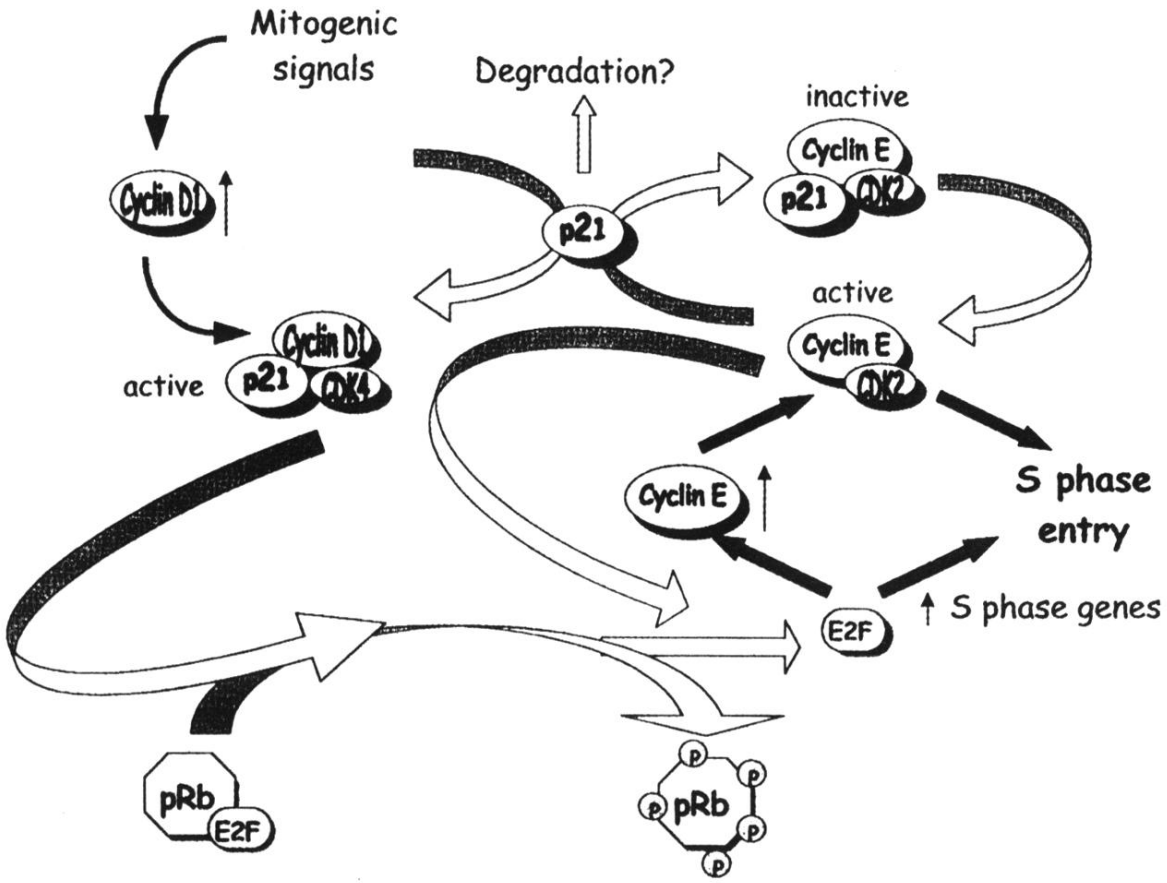


Figure 3. Regulation of G-1/S transition. In quiescent cells the levels of p27 are high, whereas p21 levels are low. Mitogenic signals during G-1 lead to an increase in p21 as well as in cyclin D by transcriptional activation. p21 (and p27) then promote the assembly of cdk-cyclin D by forming complexes with the cdk-cyclin kinases. The resulting activation of cyclin D-dependent kinases phosphorylates pRb. The sequestration of p21 (and p27) by cyclin D lowers the effective levels of p21/p27 available for inhibition of cyclin E-dependent kinase, thereby facilitating cdk2-cyclin E activation in late G-1. Ectopic expression of catalytically inactive cdk4 leads to the activation of cyclin E-dependent kinase activity, presumably by sequestering p21/p27 [140]. Activation of cdk2-cyclin E can complete the phosphorylation of pRb, leading to its inhibition and the activation of E2F transcription factor. Finally, cdk2-cyclin E phosphorylates p27 to trigger its destruction [141, 142]. These events allow cells to enter S-phase.

Model buněčného dělení stimulovaného mitogenem

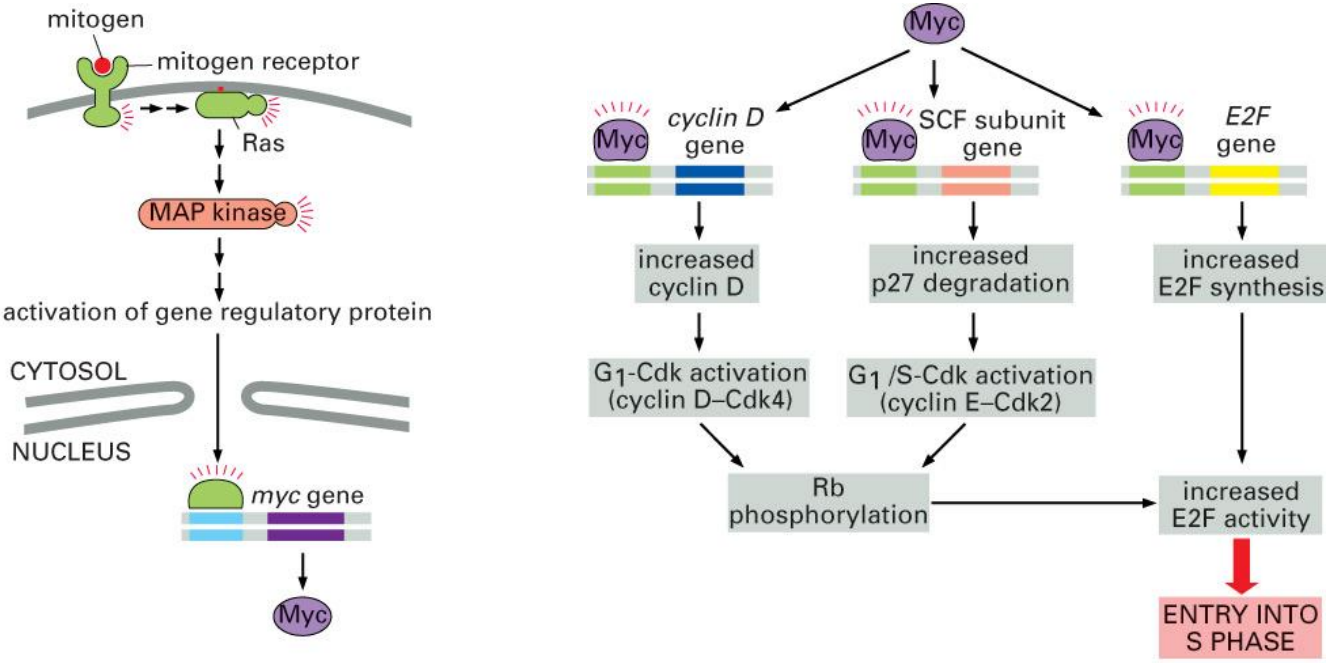
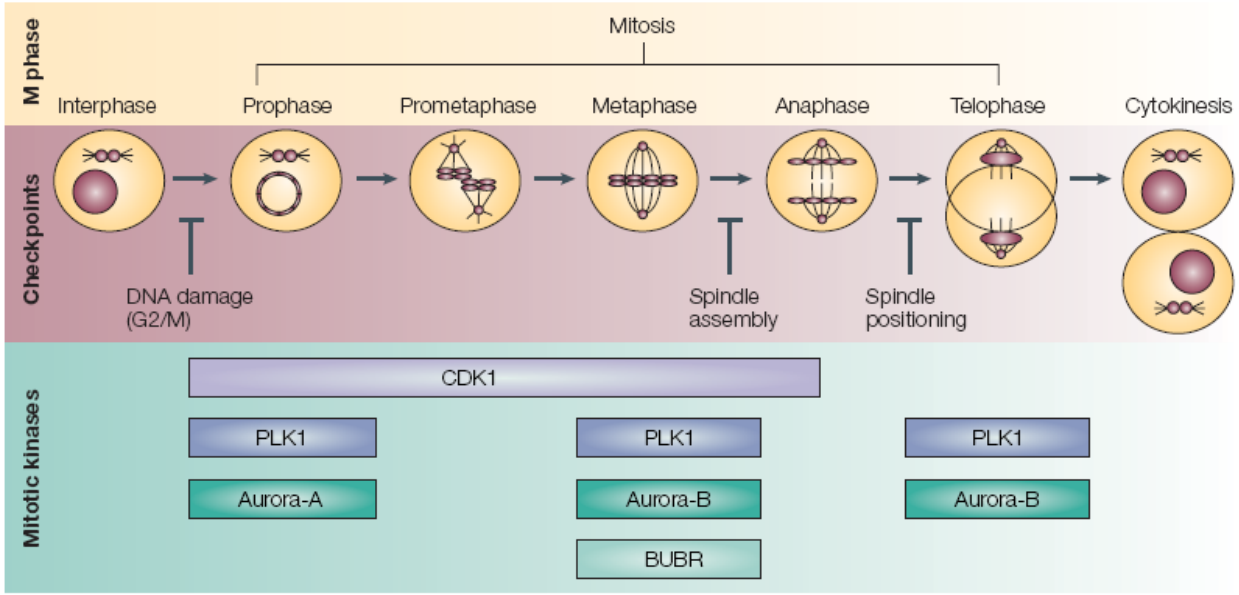


Figure 17–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Kontrolní body vstupu buněk do mitózy

Box 1 | **The M phase of the mammalian cell cycle.**

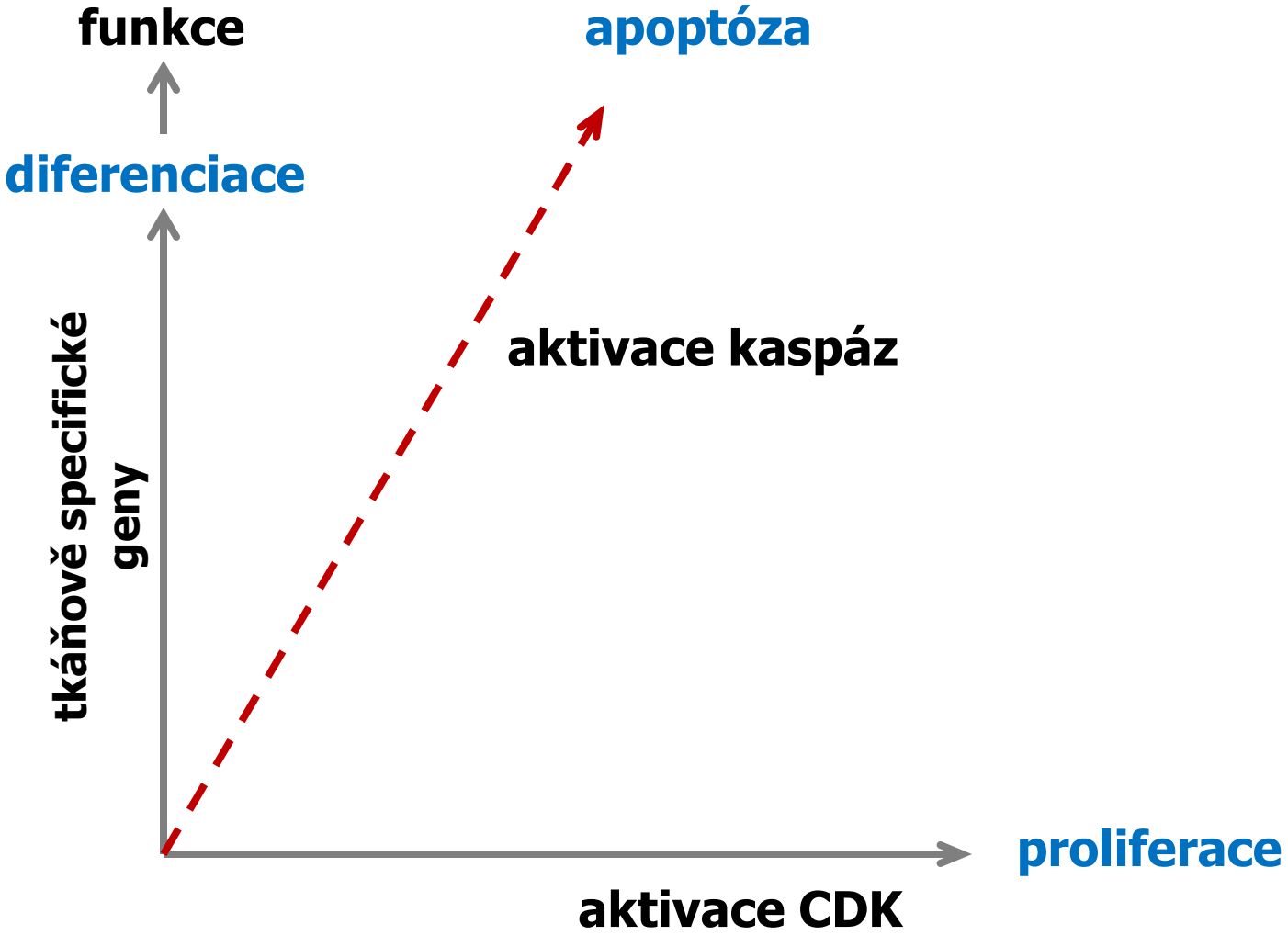
Mitosis occurs in the indicated phases to duplicate chromosomal DNA and ensure that both daughter cells receive the correct genetic complement. Cytokinesis then partitions the cytoplasmic components evenly between the daughter cells. The integrity of M-phase progression is monitored by three main checkpoints: one that detects DNA damage (G2/M arrest), one that detects mitotic-spindle malformations and one that detects incorrect positioning of the spindle. Defects in genes that are required for inducing mitotic catastrophe can contribute to tumorigenesis. These genes include those that encode the numerous kinases that are involved in mitotic regulation, such as polo-like kinase 1 (PLK1), the aurora kinase family (aurora-A and aurora-B), and a regulator of the spindle checkpoint called BUB-related kinase (BUBR). In response to genotoxic stress, ataxia teleangiectasia mutated (ATM) and ATM- and Rad3-related (ATR) become activated and, in turn, activate the checkpoint kinases CHK2 and CHK1, respectively. These kinases phosphorylate and inactivate CDC25, the molecule that is responsible for cyclin-dependent kinase 1 (CDK1) activation. When it is phosphorylated, CDC25 is translocated into the cytosol together with 14-3-3σ, and this blocks CDK1/cyclin activation, establishing G2 arrest.



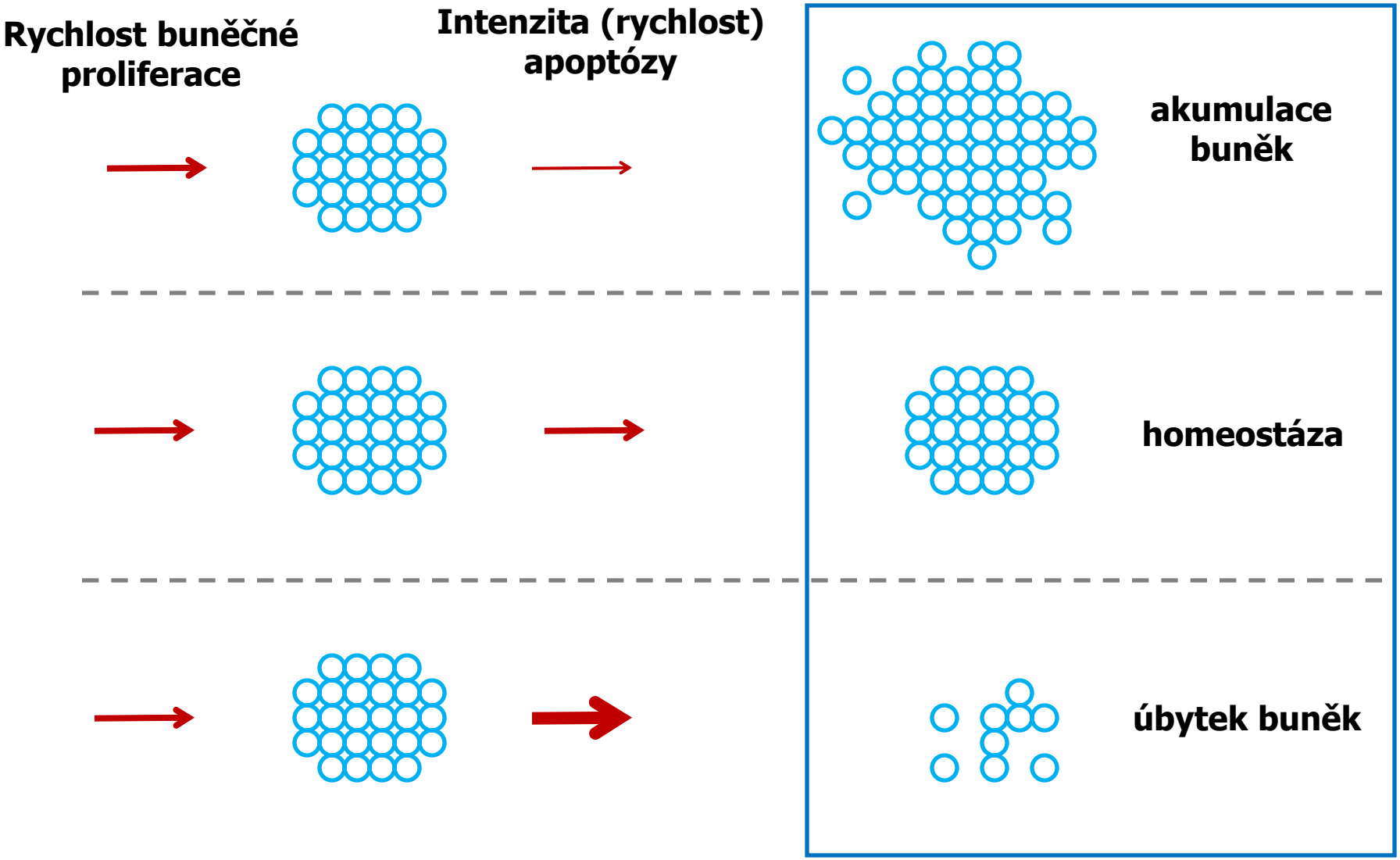
Kotrola při poškození DNA (zástava G2/M), při malformací a nesprávné pozici mitotického vřeténka. Poruchy genů indukujících „mitotickou katastrofu" (PLK1, Aurora, BUBR) mohou přispívat ke karcinogenezi.

Úloha apoptózy v karcinogenezi

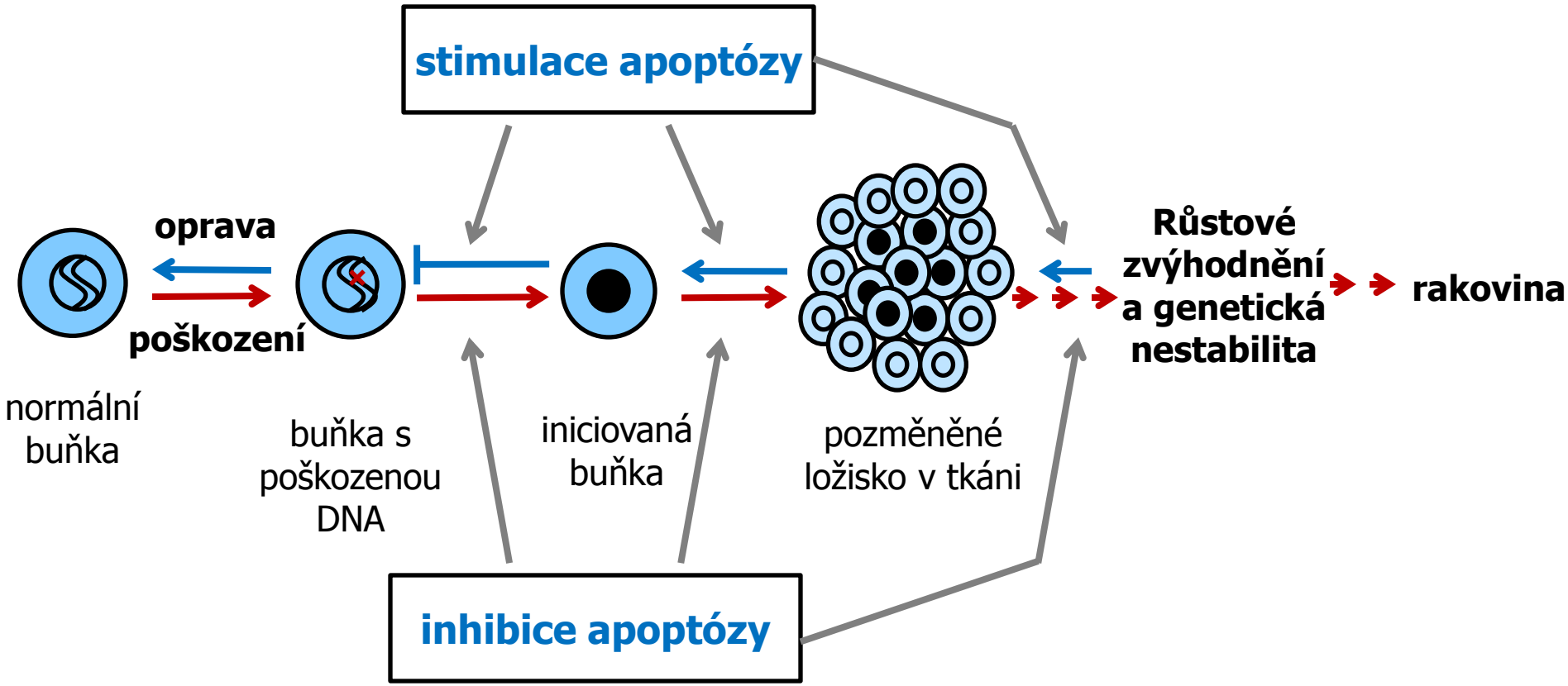
Univerzální model: trojrozměrné znázornění funkcí proliferace, apoptózy a diferenciace



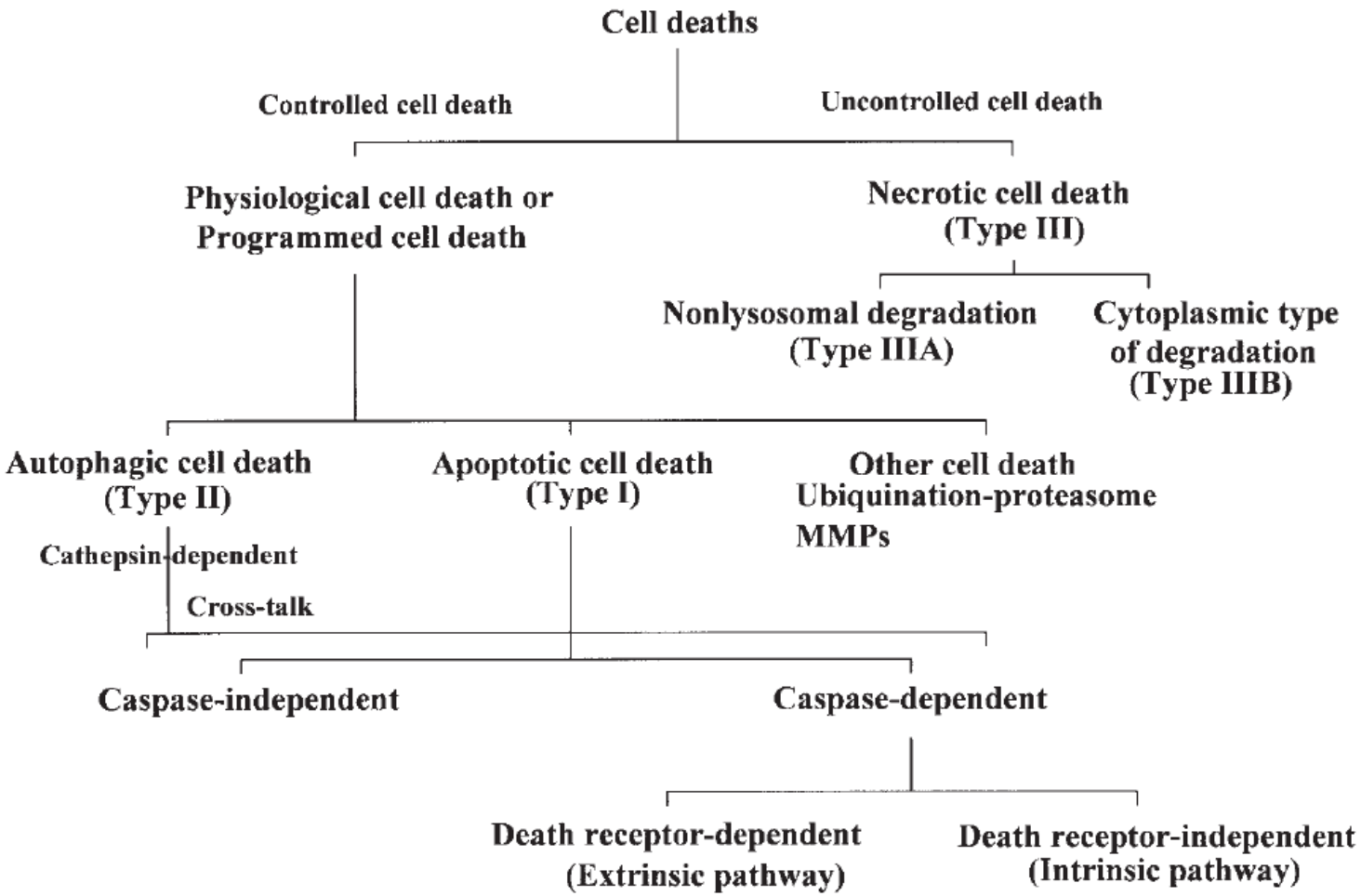
Vliv různé intenzity apoptózy na homeostázu



Vliv narušení (stimulace/inhibice) průběhu apoptózy v rámci procesu vícestupňové karcinogeneze



Různé typy buněčné smrti



Kontrolovaná (programovaná) a nekontrolovaná (nekrotická) buněčná smrt.
Programovaná bun. smrt – apoptóza a autofagie a další typy.
Apoptóza – aktivace kaspáz – možné překryvy s autofagií

Vlastnosti různých typů buněčné smrti

Table1 | **Characteristics of different types of cell death**

Type of cell death	Morphological changes			Biochemical features	Common detection methods
	Nucleus	*Cell membrane*	*Cytoplasm*		
Apoptosis	Chromatin condensation; nuclear fragmentation; DNA laddering	Blebbing	Fragmentation (formation of apoptotic bodies)	Caspase-dependent	Electron microscopy; TUNEL staining; annexin staining; caspase-activity assays; DNA-fragmentation assays; detection of increased number of cells in subG1/G0; detection of changes in mitochondrial membrane potential
Autophagy	Partial chromatin condensation; no DNA laddering	Blebbing	Increased number of autophagic vesicles	Caspase-independent; increased lysosomal activity	Electron microscopy; protein-degradation assays; assays for marker-protein translocation to autophagic membranes; MDC staining
Mitotic catastrophe	Multiple micronuclei; nuclear fragmentation	–	–	Caspase-independent (at early stage) abnormal CDK1/cyclin B activation	Electron microscopy; assays for mitotic markers (MPM2); TUNEL staining
Necrosis	Clumping and random degradation of nuclear DNA	Swelling; rupture	Increased vacuolation; organelle degeneration; mitochondrial swelling	–	Electron microscopy; nuclear staining (usually negative); detection of inflammation and damage in surrounding tissues
Senescence	Distinct heterochromatic structure (senescence-associated heterochromatic foci)	–	Flattening and increased granularity	SA-β-gal activity	Electron microscopy; SA-β-gal staining; growth-arrest assays; assays for increased p53, INK4A and ARF levels (usually increased); assays for RB phosphorylation (usually hypophosphorylated); assays for metalloproteinase activity (usually upregulated)

CDK1, cycline-dependent kinase 1; MDC, monodansylcadaverine; MPM2, mitotic phosphoprotein 2; SA-β-gal, senescence-associated β-galactosidase; RB, retinoblastoma protein.

Okada H. and Mak TW, Nature Rev Cancer 2004

Rozdílné vlastnosti apoptózy a autofagie

TABLE 1
Differences in Characteristics between Type I and Type II Cell Death

Factor	Apoptosis (type I)	Autophagy (type II)
Morphology		
Nucleus	Chromatin condensation	Partial chromatin condensation
	DNA laddering and fragmentation	No DNA laddering and fragmentation
	Pyknosis	Sometimes pyknosis
Cytoplasm	Cytoplasmic condensation	Many large autophagic vacuoles
	Fragmentation to apoptotic bodies	Many autophagosomes
	Increase in MMP	Lysosomal activation
	Activation of caspase cascade	Caspase independent
	Potential release of lysosomal enzyme	Potential involvement of MMP
	Organelles do not swell	
Membrane	Blebbing	Blebbing
Primary proteases	Caspases such as caspase 3	Cathepsins and proteasomal proteins
ATP requirement	Yes	Yes (AMPK, AMP/ATP ratio)
Inhibition	z-VAD-fmk	3-methyladenine (3-MA)
	XIAP	PI3K inhibitors
	Bcl-2/Bcl-xL	PI3K-I/Akt
	Sometimes with actinomycin D	Actinomycin D
	Sometimes with cyclophosphamide	Cyclophosphamide
Detection	DNA laddering test	Electron microscopy
	Caspase activation and the substrate	Lysosome activity test
	TUNEL and annexin V staining	Cytoplasmic sequestration test
	FACS analysis	LC3 associated with autophagosome membrane
	Electron microscopy	

MMP: mitochondrial membrane permeability; XIAP: X chromosome-encoded inhibitors of apoptosis proteins; PI3K: phosphoinositide 3-kinase; TUNEL: terminal deoxynucleotidyl transferase nick-end labeling; FACS: fluorescence-activated cell sorting; AMPK: AMP-activated protein kinase; AMP: adenosine 5′-monophosphate; ATP: adenosine 5′-triphosphate.

Vztah apoptózy a autofagie

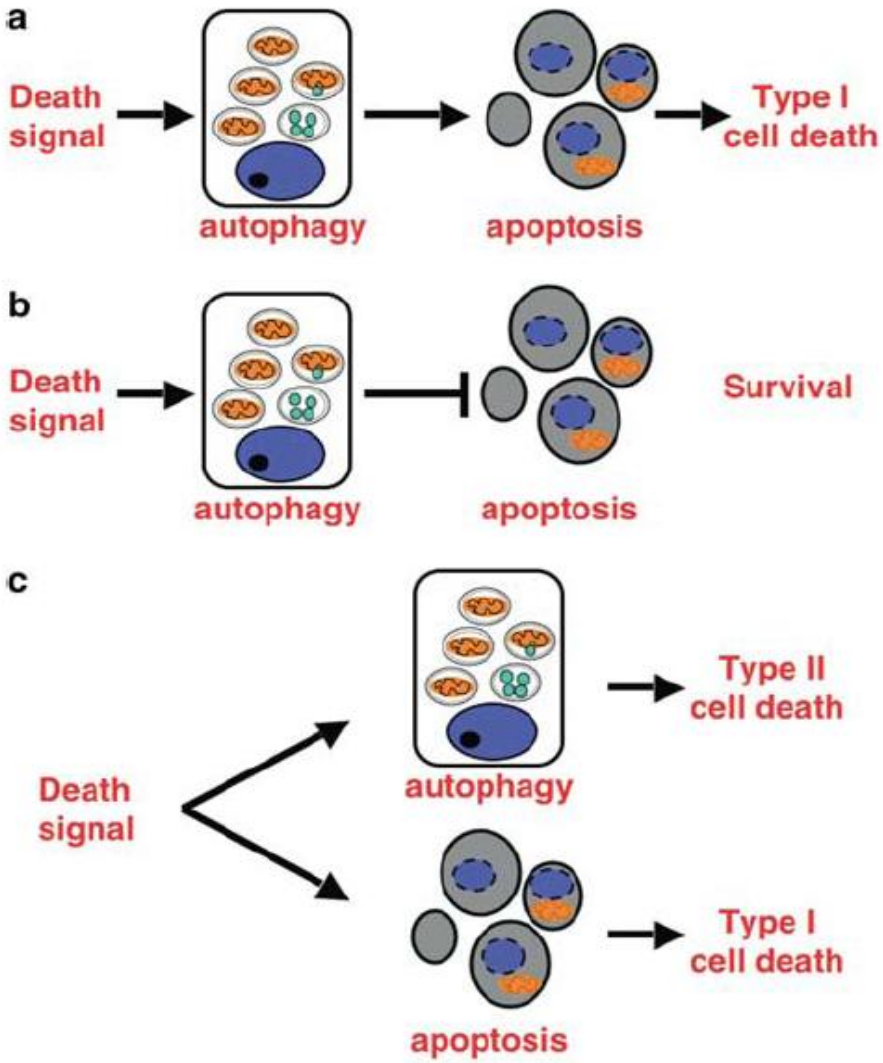
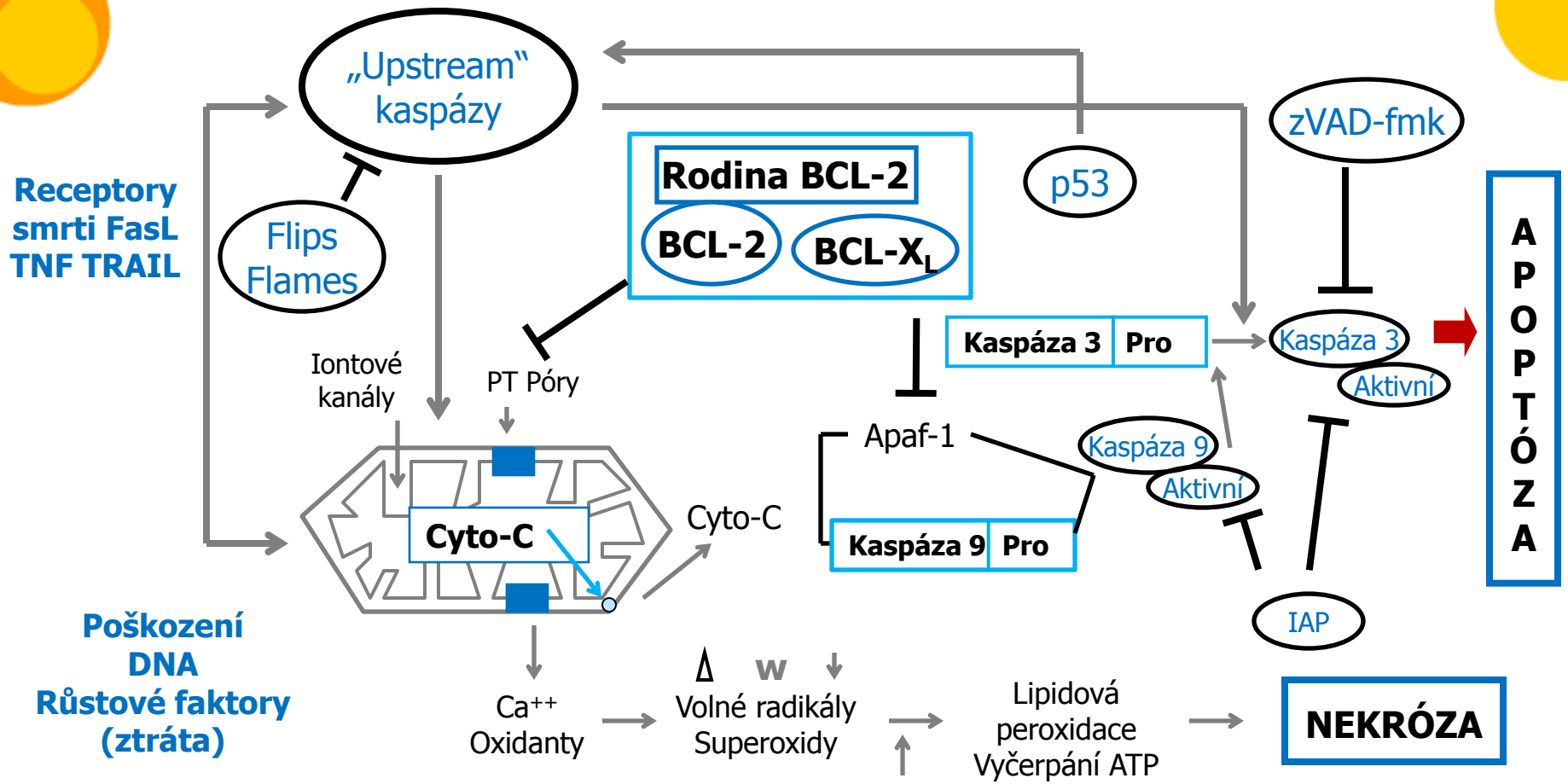


Figure 4 Apoptosis/autophagy connection in programmed cell death context. (**a**) Autophagy may be indispensable for apoptosis occurrence. (**b**) Autophagy may antagonize apoptosis (**c**) Apoptosis and autophagy may occur independent of each other. Inhibition of apoptosis may convert cell death morphology to autophagic and *vice versa*

Autofagie může být nezbytná pro apoptózu či působit proti ní.

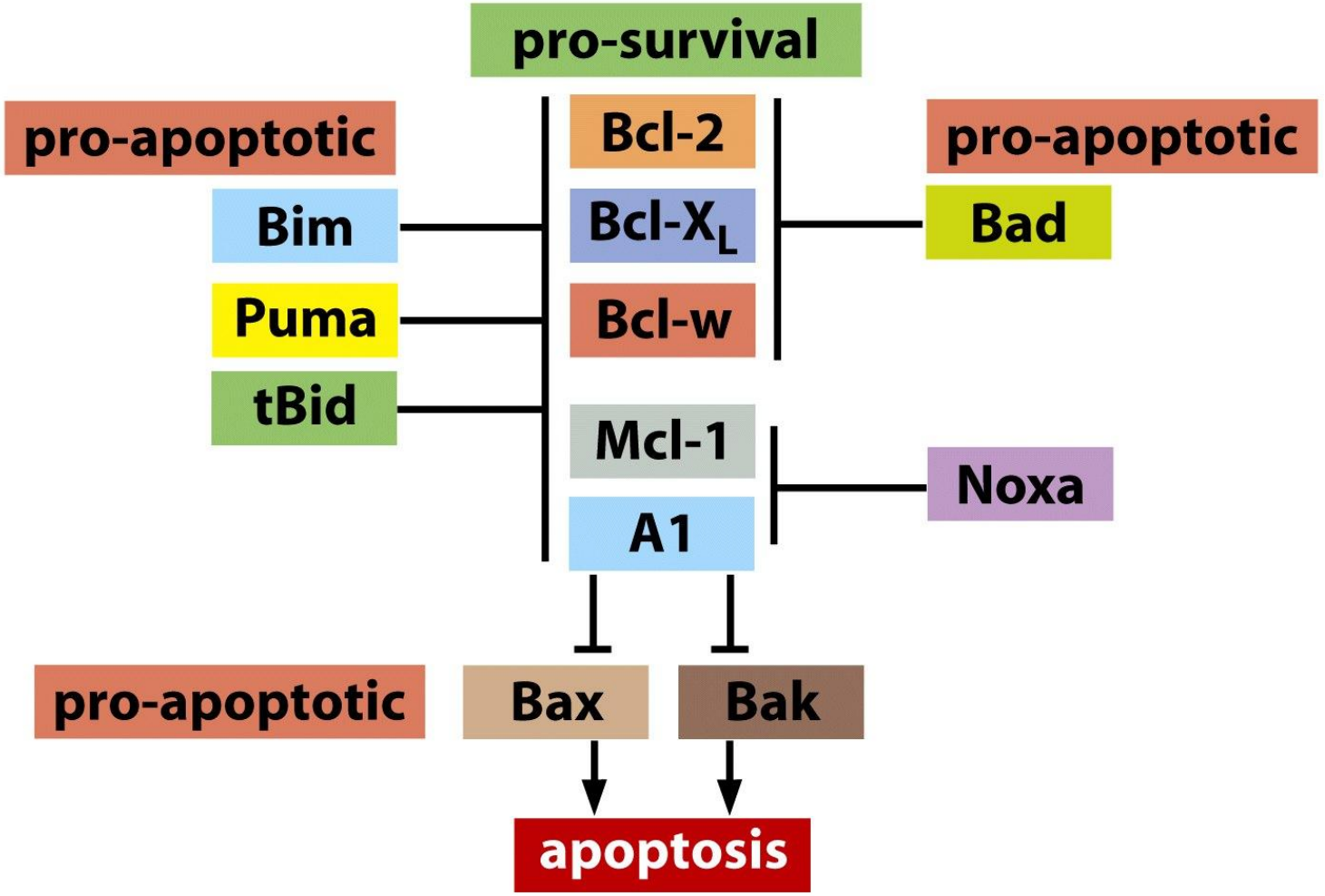
Autofagie a apoptóza mohou rovněž existovat nezávisle na sobě.

Inhibice apoptózy může zvrátit bun. smrt v autofagii a naopak.



Dráhy kontrolující apoptózu a nekrózu. Aktivace receptorů smrti (DRs), poškození DNA ztráta růstových faktorů, radio- nebo chemoterapie mohou vyústit v aktivaci „upstream" kaspáz, aktivaci mitochondrií, uvolnění cytochromu c, aktivaci Apaf-1, následnou aktivaci „downstream" kaspáz a konečně ve fragmentaci DNA a apoptózu. Klíčovou roli hrají anti-apoptotické členy rodiny Bcl-2 (Bcl-2, Bcl-X_L) a inhibitory jako IAP (inhibitory apoptických proteinů). Mitochondriální aktivace vyúsťuje v uvolnění Ca^{++}, tvorbu volných radikálů, peroxidaci lipidů a vyčerpání ATP, což může vést k nekróze.

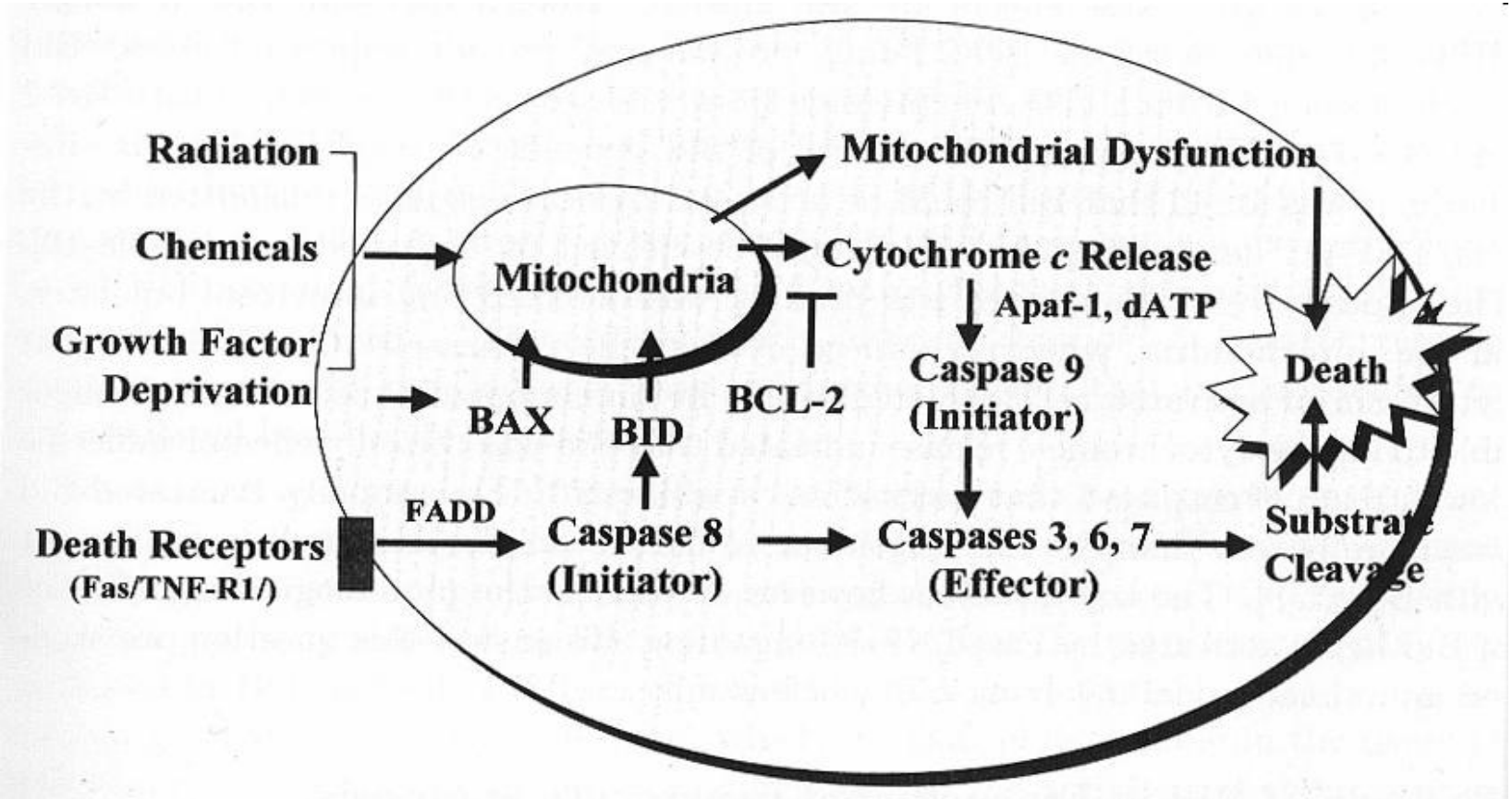
Rodina proteinů Bcl-2



Důležitá je rovnováha proapoptoticky a antiapoptoticky působících proteinů

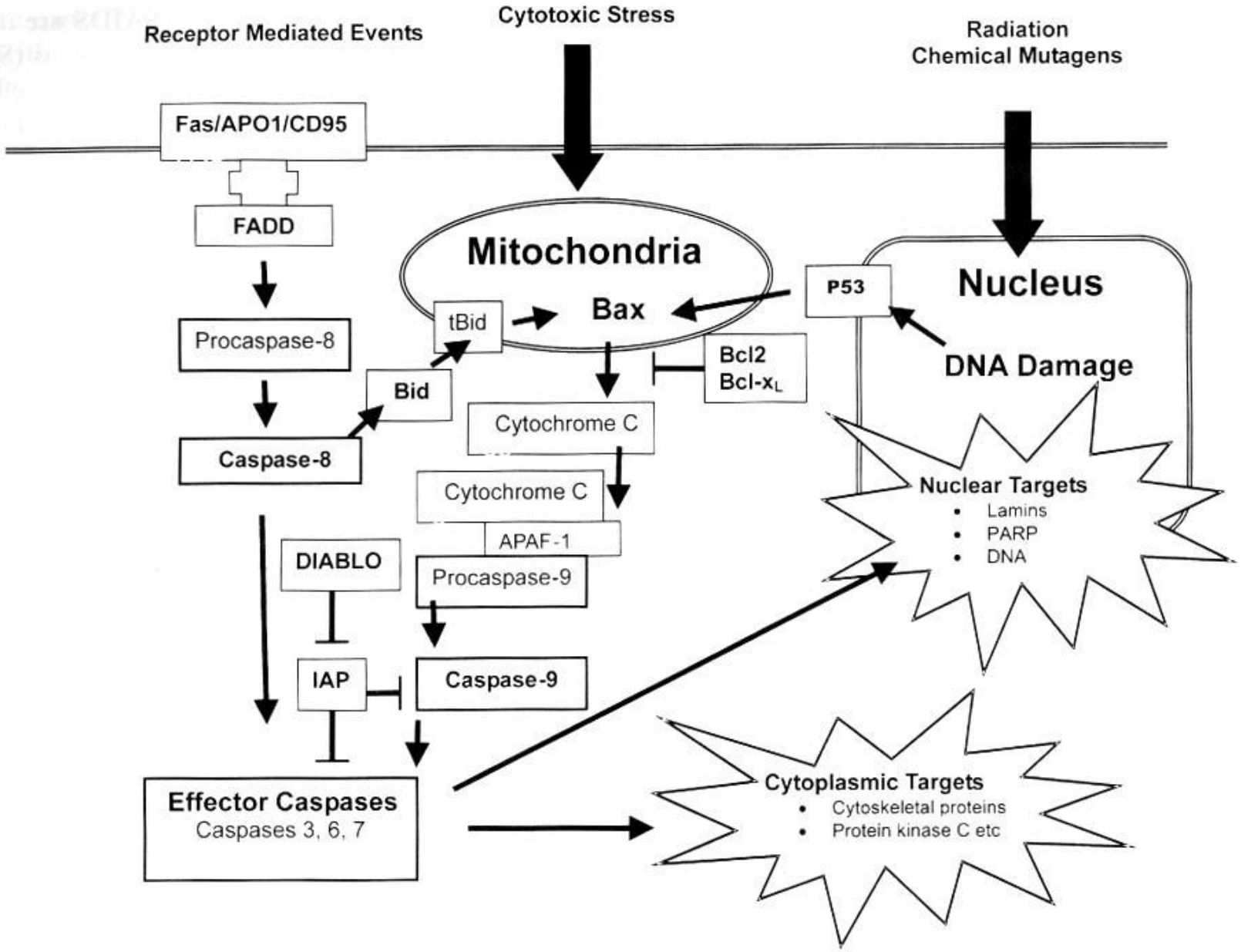
Figure 9.27c *The Biology of Cancer* (© Garland Science 2007)

Různé podněty indukující apoptózu buněk



Indukovat apoptózu může záření, různé chemikálie (vč. léčiv), nepřítomnost růstových a viabilitních faktorů, specifické cytokiny aktivující receptory smrti (death receptors –DR)

Iniciace a regulace apoptózy po různých podnětech



Vnitřní (mitochondriální, intrinsic) a vnější (extrinsic) dráha apoptózy

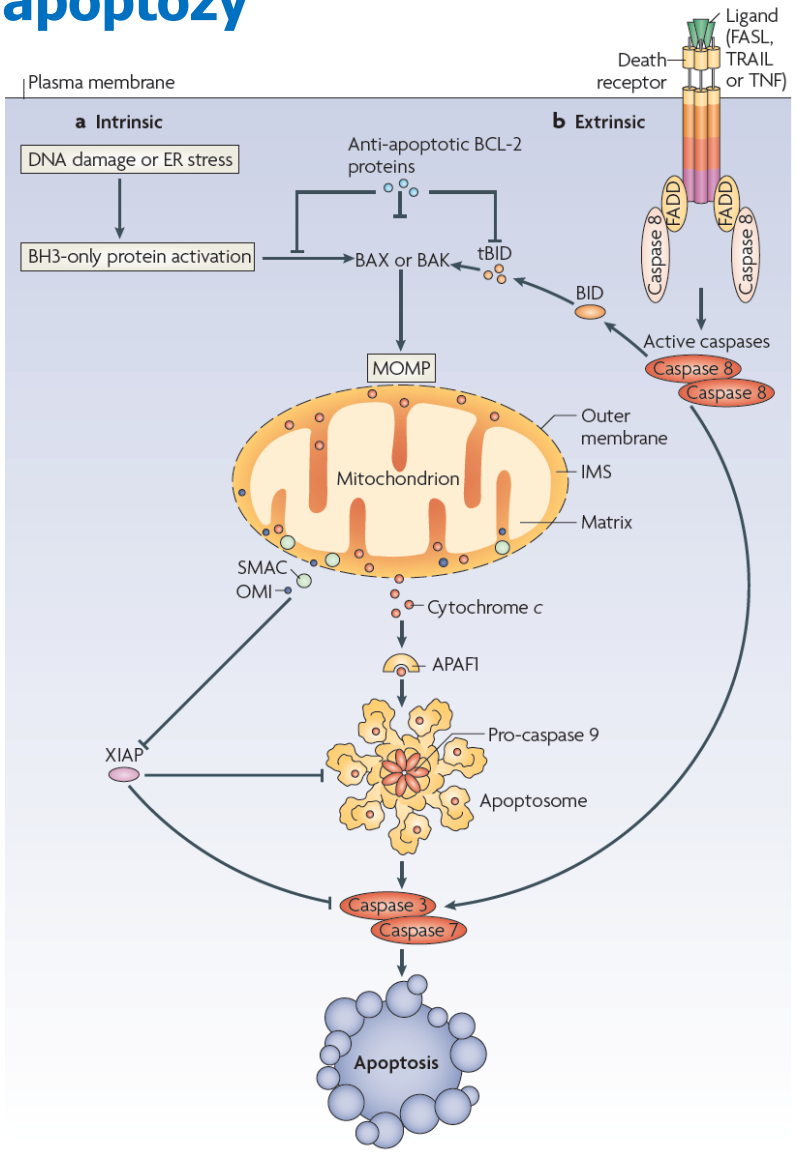


Figure 1 | **Intrinsic and extrinsic pathways of apoptosis. a** | Intrinsic apoptotic stimuli, such as DNA damage or endoplasmic reticulum (ER) stress, activate B cell lymphoma 2 (BCL-2) homology 3 (BH3)-only proteins leading to BCL-2-associated X protein (BAX) and BCL-2 antagonist or killer (BAK) activation and mitochondrial outer membrane permeabilization (MOMP). Anti-apoptotic BCL-2 proteins prevent MOMP by binding BH3-only proteins and activated BAX or BAK. Following MOMP, release of various proteins from the mitochondrial intermembrane space (IMS) promotes caspase activation and apoptosis. Cytochrome *c* binds apoptotic protease-activating factor 1 (APAF1), inducing its oligomerization and thereby forming a structure termed the apoptosome that recruits and activates an initiator caspase, caspase 9. Caspase 9 cleaves and activates executioner caspases, caspase 3 and caspase 7, leading to apoptosis. Mitochondrial release of second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) and OMI (also known as HTRA2) neutralizes the caspase inhibitory function of X-linked inhibitor of apoptosis protein (XIAP). **b** | The extrinsic apoptotic pathway is initiated by the ligation of death receptors with their cognate ligands, leading to the recruitment of adaptor molecules such as FAS-associated death domain protein (FADD) and then caspase 8. This results in the dimerization and activation of caspase 8, which can then directly cleave and activate caspase 3 and caspase 7, leading to apoptosis. Crosstalk between the extrinsic and intrinsic pathways occurs through caspase 8 cleavage and activation of the BH3-only protein BH3-interacting domain death agonist (BID), the product of which (truncated BID; tBID) is required in some cell types for death receptor-induced apoptosis. FASL, FAS ligand; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

Tait SW and Green DR Nature Rev Mol Cell Biol 2010

Mitochondrální apoptická dráha

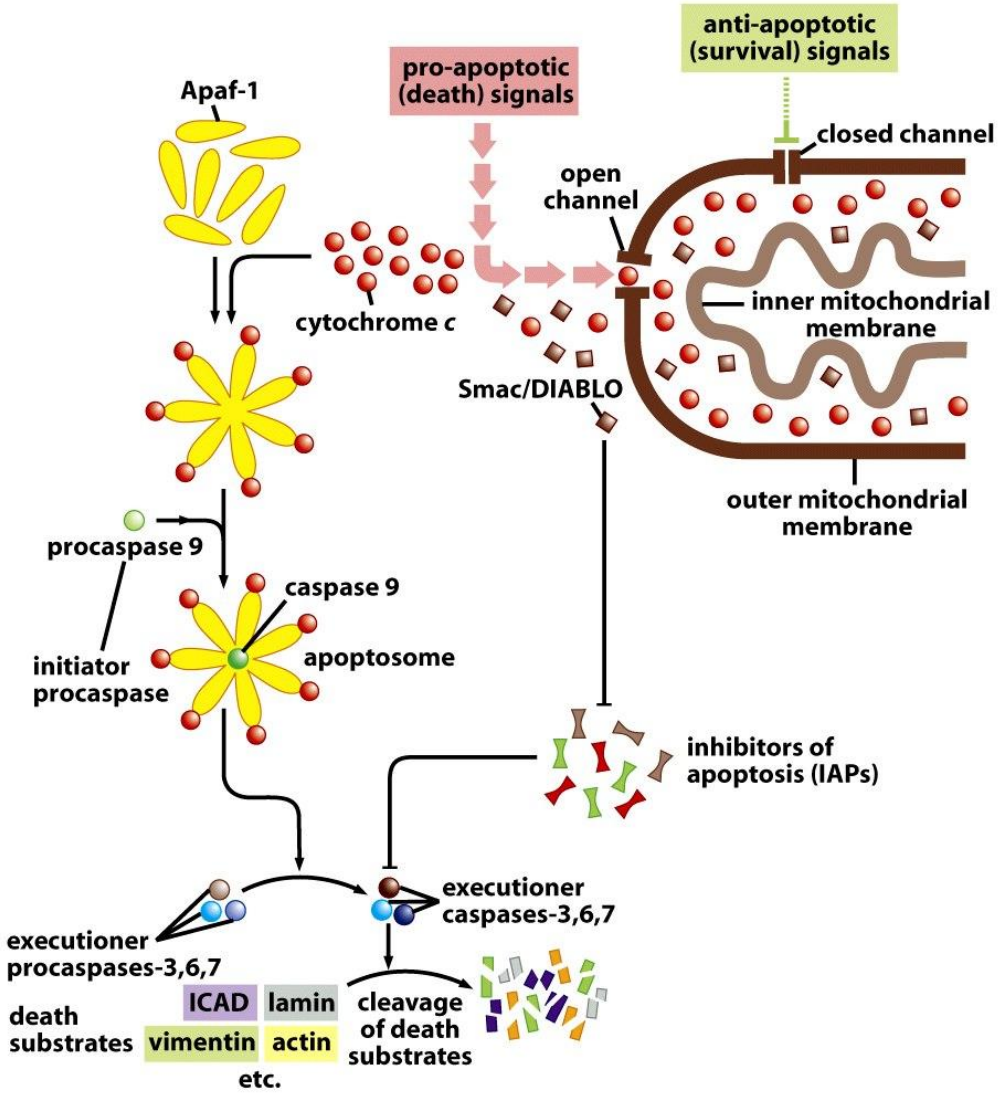
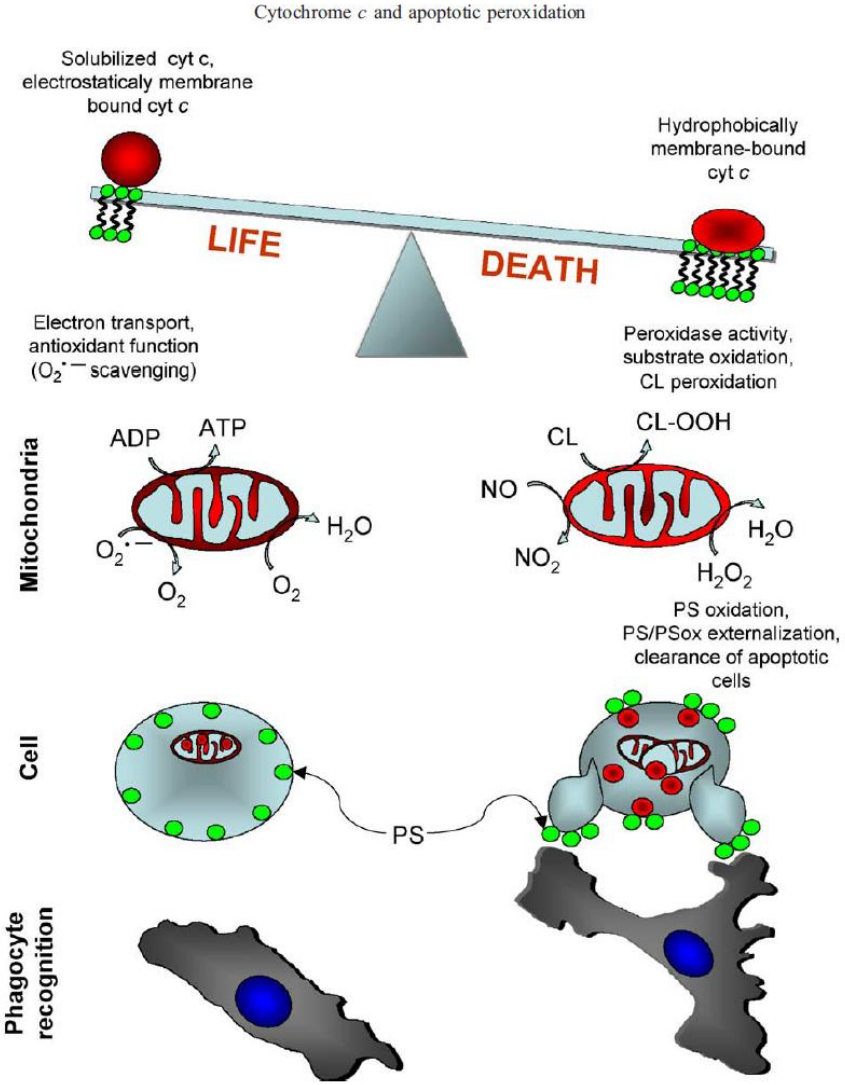


Figure 9.29 *The Biology of Cancer* (© Garland Science 2007)

Funkce cytochromu c u normální a apoptické buňky.



Scheme 2. Normal functions of cyt *c* in normally functioning cells and apoptotic cells. Redox catalysis of oxidation of anionic phospholipids—CL in mitochondria and PS in plasma membrane—triggering the formation of MPTP and PS-signaling pathways, respectively.

Apoptóza

Oxidace anionických fosfolipidů

Kardiolipin (CL)

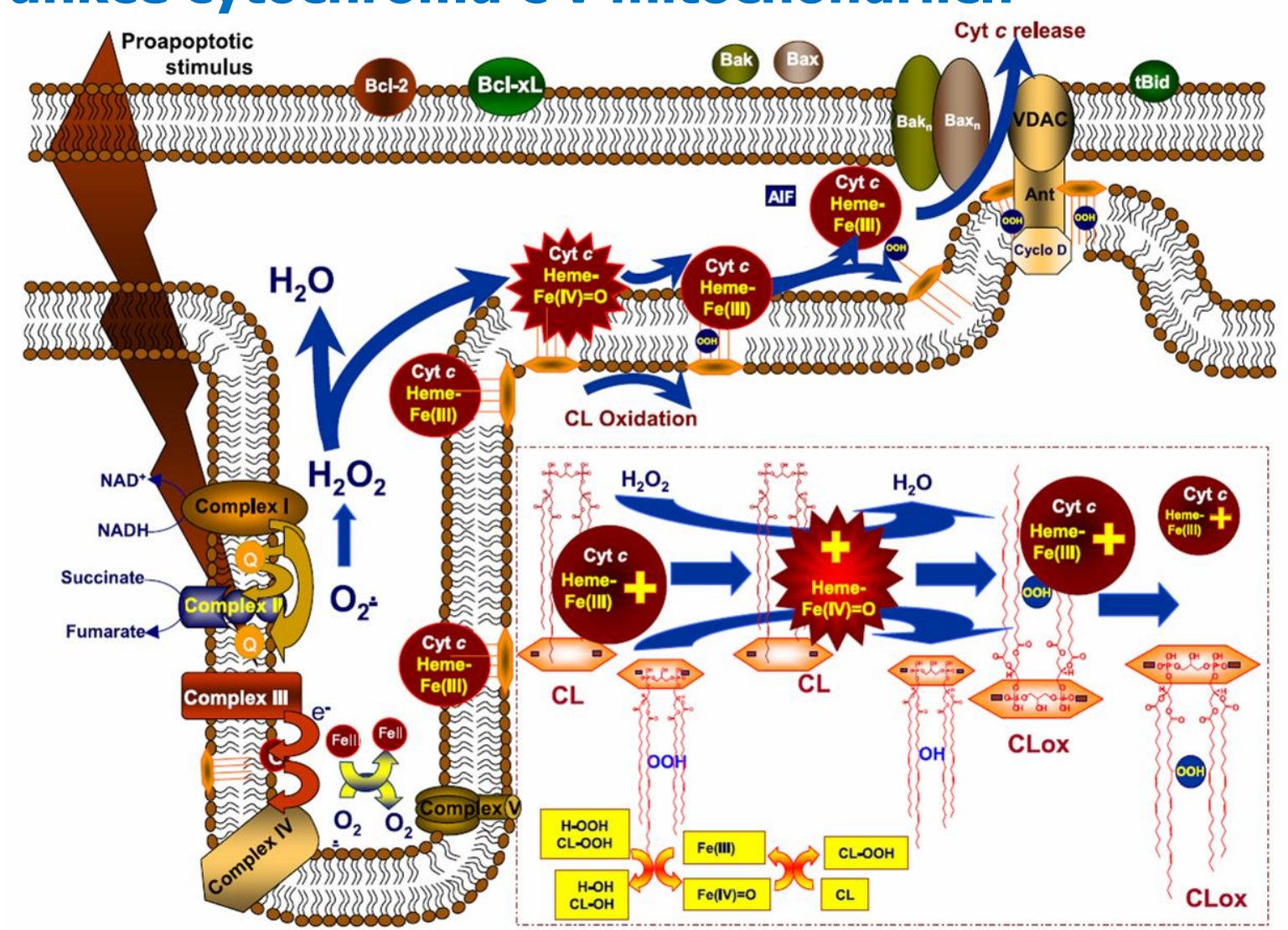
v mitochondriální membráně - tvorba specifických pórů

Fosfatidylserin (PS)

v plazmatické membráně – Externalizace PS – rozpoznání apoptických buněk fagocyty

Kagan VE et al. Free Rad Biol Med 2004

Funkce cytochromu c v mitochondriích



Scheme 1. Schematic representation of cyt *c* functions in mitochondria: pools participating in electron transport and acting as a peroxidase activated during apoptosis are shown. Insert depicts the formation of oxoferryl Fe(IV)═O form of cyt *c* by H_2O_2 or CL hydroperoxides (CL-OOH).

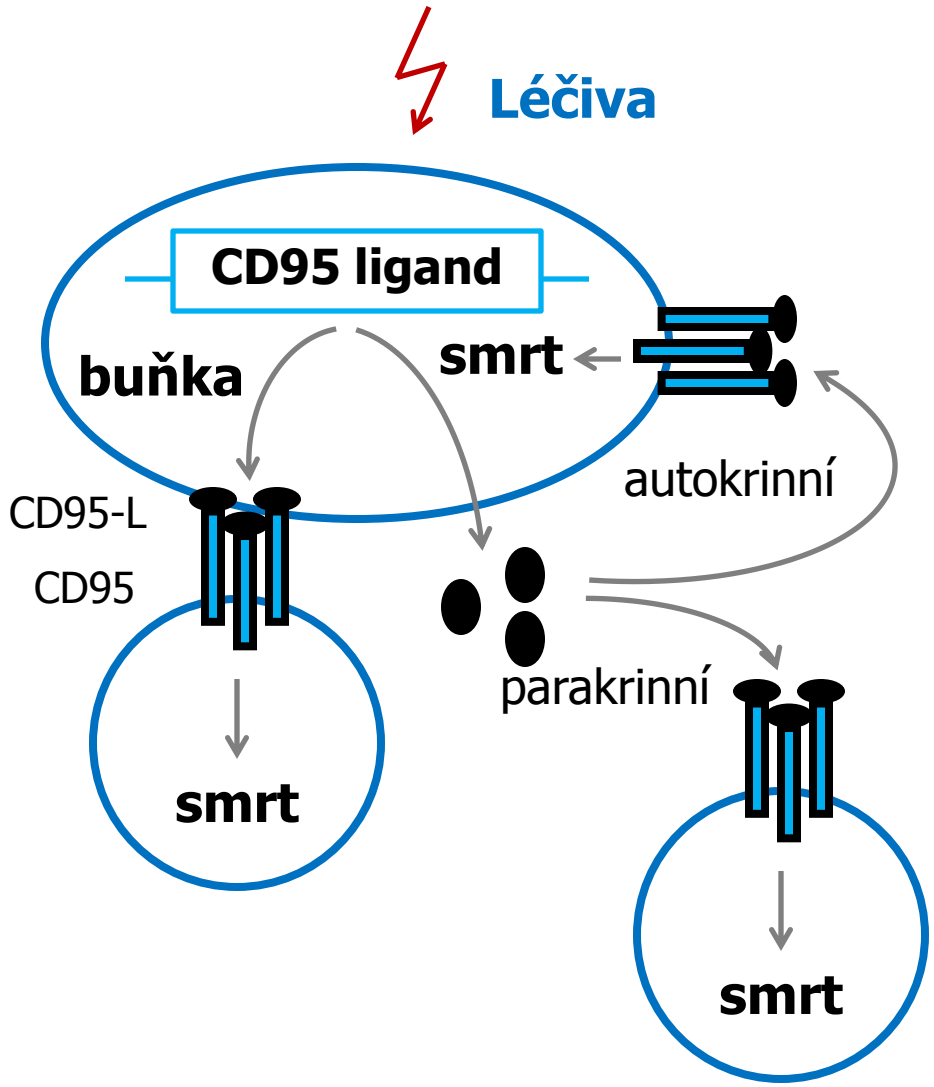
Kagan VE et al. Free Rad Biol Med 2004

Receptory smrti (DRs) a jejich ligandy

Table 9.4 Death receptors and their ligands

Alternative names of receptors	Alternative names of ligands
Fas/APO-1/CD95	FasL/CD95L
TNFR1	TNF-α
DR3/APO-3/SWL-1/TRAMP	APO3L
DR4/TRAIL-R1	APO2L/TRAIL
DR5/TRAIL-R2/KILLER	APO2L/TRAIL

Protinádorová léčiva aktivují dráhu CD95



Aktivace, inhibice a interakce signálních drah po působení růstových faktorů, buněčného stresu, poškození DNA a induktorů apoptózy

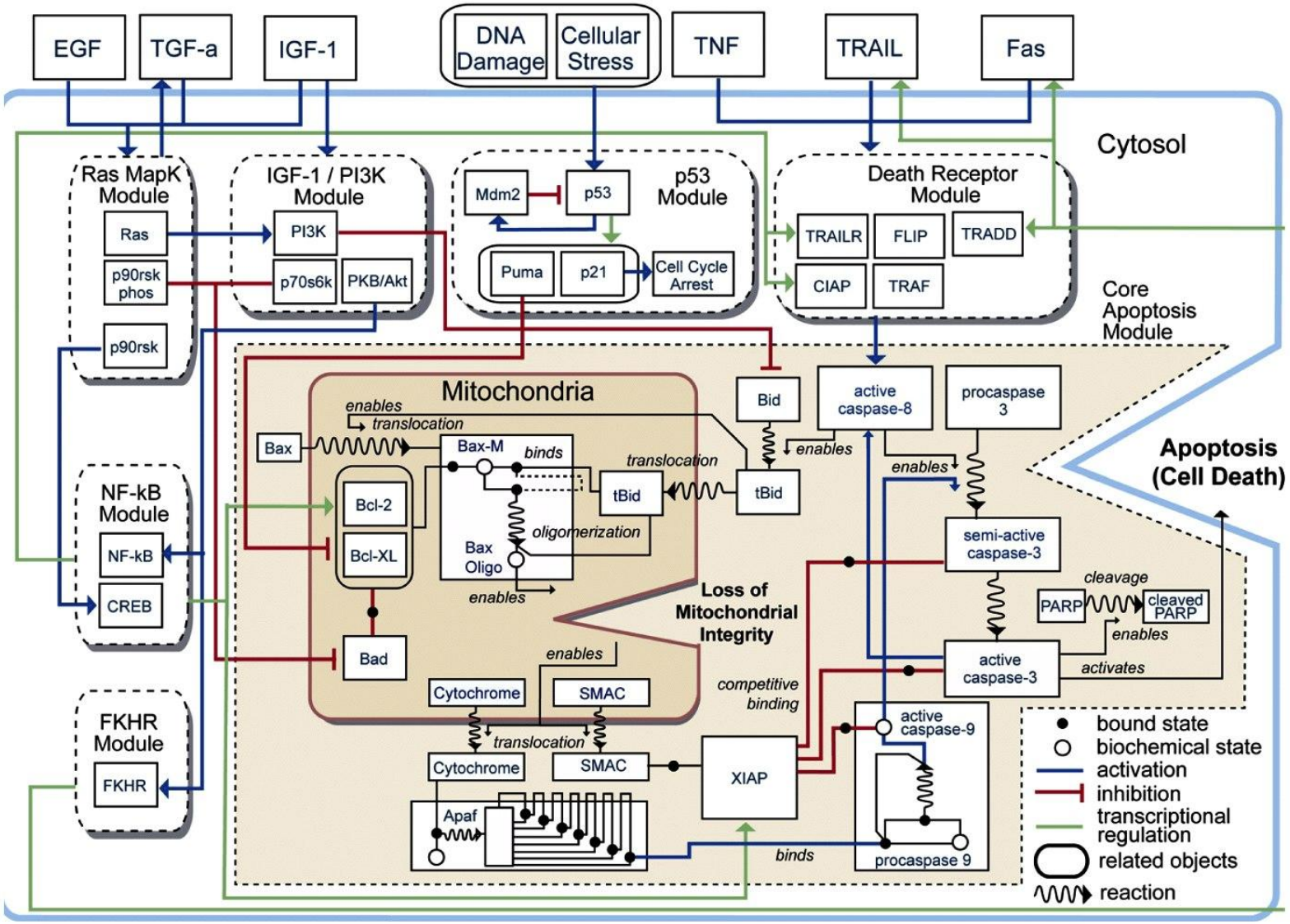
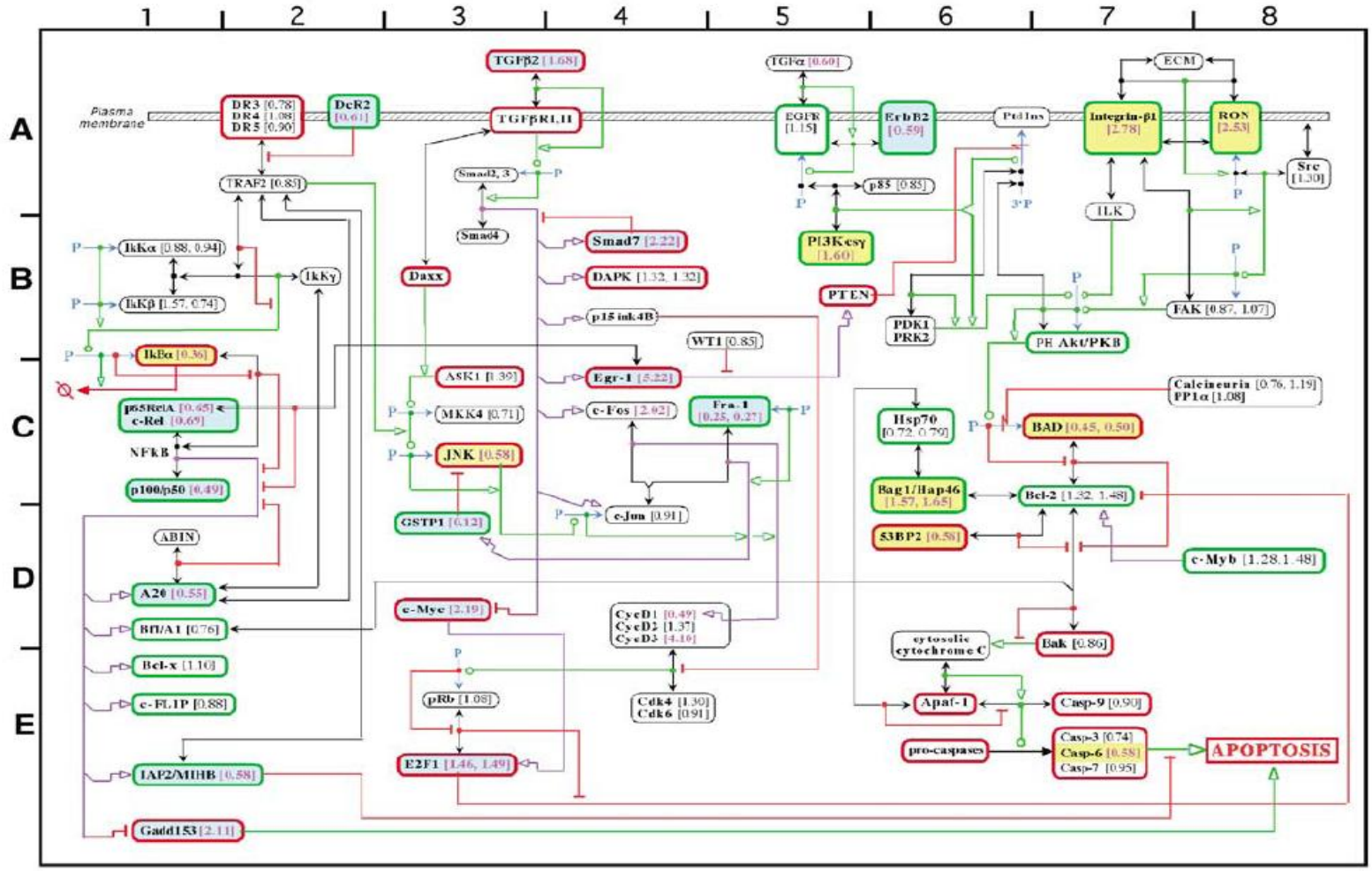


Figure 9.37 *The Biology of Cancer* (© Garland Science 2007)



Molekulární interakční mapa drah spojených s apoptózou, u nichž byly pozorovány rozdíly v genové expresi.

Molekuly podporující apoptózu – červená

Molekuly potlačující apoptózu – zelená

Exprese mRNA se mění očekávaným směrem – žlutě, opačným směrem - modře

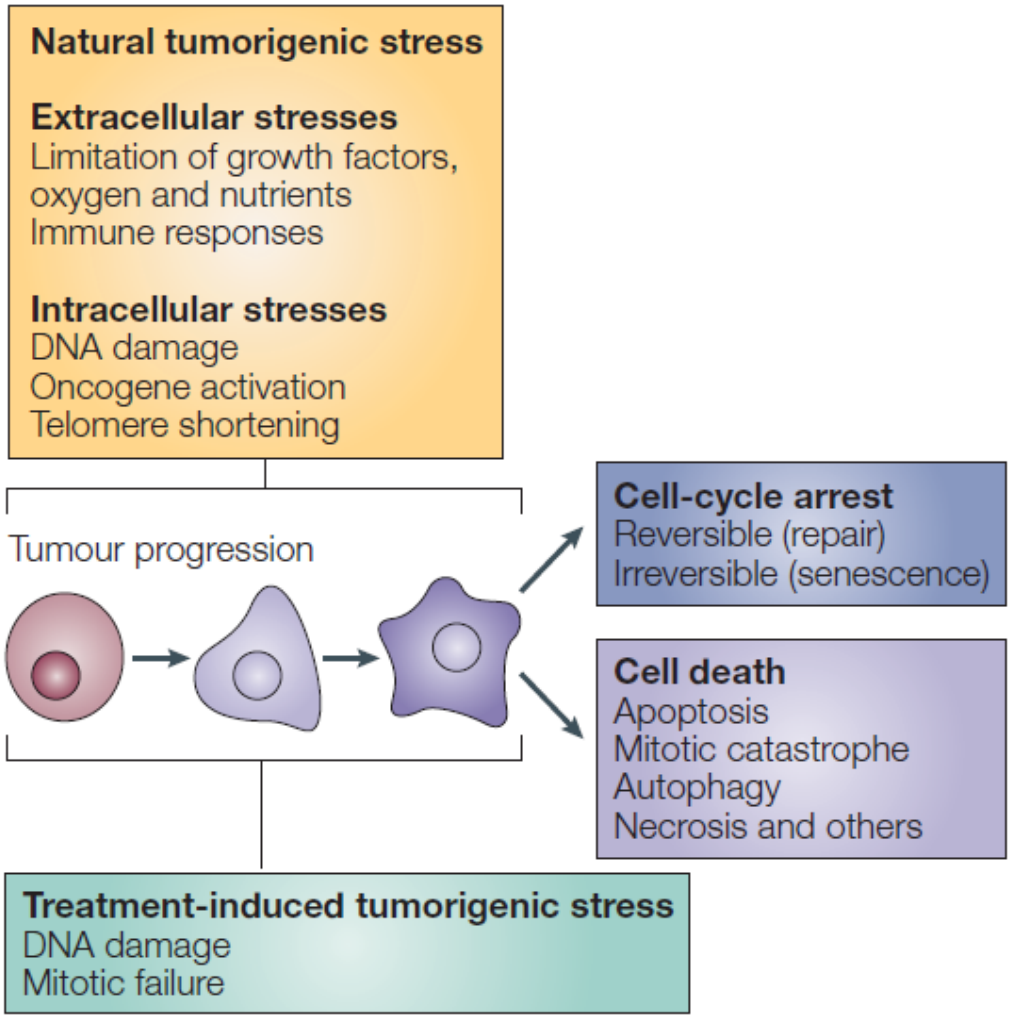
Příklady antiapoptických změn v lidských nádorech

Table 9.5 Examples of anti-apoptotic alterations found in human tumor cells

Alteration	Mechanism of anti-apoptotic action	Types of tumors
CASP8 promoter methylation	inactivation of extrinsic cascade	SCLC, pediatric tumors
CASP3 repression	inactivation of executioner caspase	breast carcinomas
Survivin overexpression[a]	caspase inhibitor	mesotheliomas, melanomas, many carcinomas
ERK activation	repression of caspase-8 expression	many types
ERK activation	protection of Bcl-2 from degradation	many types
Raf activation	sequestration of Bad by 14-3-3 proteins	many types
PI3K mutation/activation	activation of Akt/PKB	gastrointestinal
NF-κB constitutive activation[b]	induction of anti-apoptotic genes	many types
p53 mutation	loss of ability to induce pro-apoptotic genes	many types
$p14^{ARF}$ gene inactivation	suppression of p53 levels	many types
Mdm2 overexpression	suppression of p53 levels	sarcomas
IAP-1 gene amplification	antagonist of caspases-3 and 7	esophageal, cervical
APAF1 methylation	loss of caspase-9 activation by cytochrome *c*	melanomas
BAX mutation	loss of pro-apoptotic protein	colon carcinomas
Bcl-2 overexpression	closes mitochondrial channel	~ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma
IGF-1/2 overexpression	activates PI3K	many types
IGFBP repression	loss of anti-apoptotic IGF-1/2 antagonist	many types
Casein kinase II	activation of NF-κB	many types
TNFR1 methylation	repressed expression of death receptor	Wilms tumor
FLIP overexpression	inhibition of caspase-8 activation by death receptors	melanomas, many others
Akt/PKB activation	phosphorylation and inactivation of pro-apoptotic Bcl-2-like proteins	many types
Stat3 activation	induces expression of $Bcl\text{-}X_L$	several types
TRAIL-R1 repression	loss of responsiveness to death ligand	small-cell lung carcinoma
FAP-1 overexpression	inhibition of Fas receptor signaling	pancreatic carcinoma
XAF1 methylation[c]	loss of inhibition of anti-apoptotic XIAP	gastric carcinoma
Wip1 overexpression[d]	suppression of p53 activation	breast and ovarian carcinomas, neuroblastoma

[a]Survivin is an inhibitor of apoptosis (IAP) in gastric, lung, and bladder cancer and melanoma in addition to the mesotheliomas indicated here. The expression of a number of IAP genes is directly induced by the NF-κB TFs.
[b]Induces synthesis of c-IAPs, XIAP, $Bcl\text{-}X_L$, and other anti-apoptotic proteins.
[c]XAF1 (XIAP-associated factor 1) normally binds and blocks the anti-apoptotic actions of XIAP, the most potent of the IAPs.
[d]Wip1 is a phosphatase that inactivates p38 MAPK, which otherwise would phosphorylate and stimulate the pro-apoptotic actions of p53.

Vnější i vnitřní stres indukuje přeměnu normální buňky v nádorovou



Vnější stres – omezení růstových faktorů, kyslíku a živin, imunitní odpověď

Vnitřní stres – poškození DNA, aktivace onkogenů, zkracování telomer

Mohou být navozeny přirozeným způsobem nebo působením nějakých látek či faktorů.

Obrana buňky – zástava buněčného cyklu, reparace, senescence, buněčná smrt

Figure 1 | **Responses of cells to natural and treatment-induced tumorigenic stresses.** Both extracellular and intracellular stresses — generated by natural means or by treatment — can induce tumorigenic changes in normal cells. Cells then attempt to avoid transformation by undergoing either cell-cycle arrest or cell death. Cell-cycle arrest allows time for the repair of DNA damage. If repair is successful, the arrest is reversed and the cell divides. If the repair is unsuccessful, the cell survives, but becomes senescent and does not divide. If the repair is unsuccessful and the cell does not become senescent, cell death must be induced to prevent tumorigenesis. Several pathways, which involve some of the same signalling mediators, exist to carry out this function, including those that lead to apoptosis, mitotic catastrophe, autophagy and necrosis.

Okada H. and Mak TW, Nature Rev Cancer 2004

Regulace apoptózy zprostředkované p53 v odpovědi na stresový signál *in vivo*

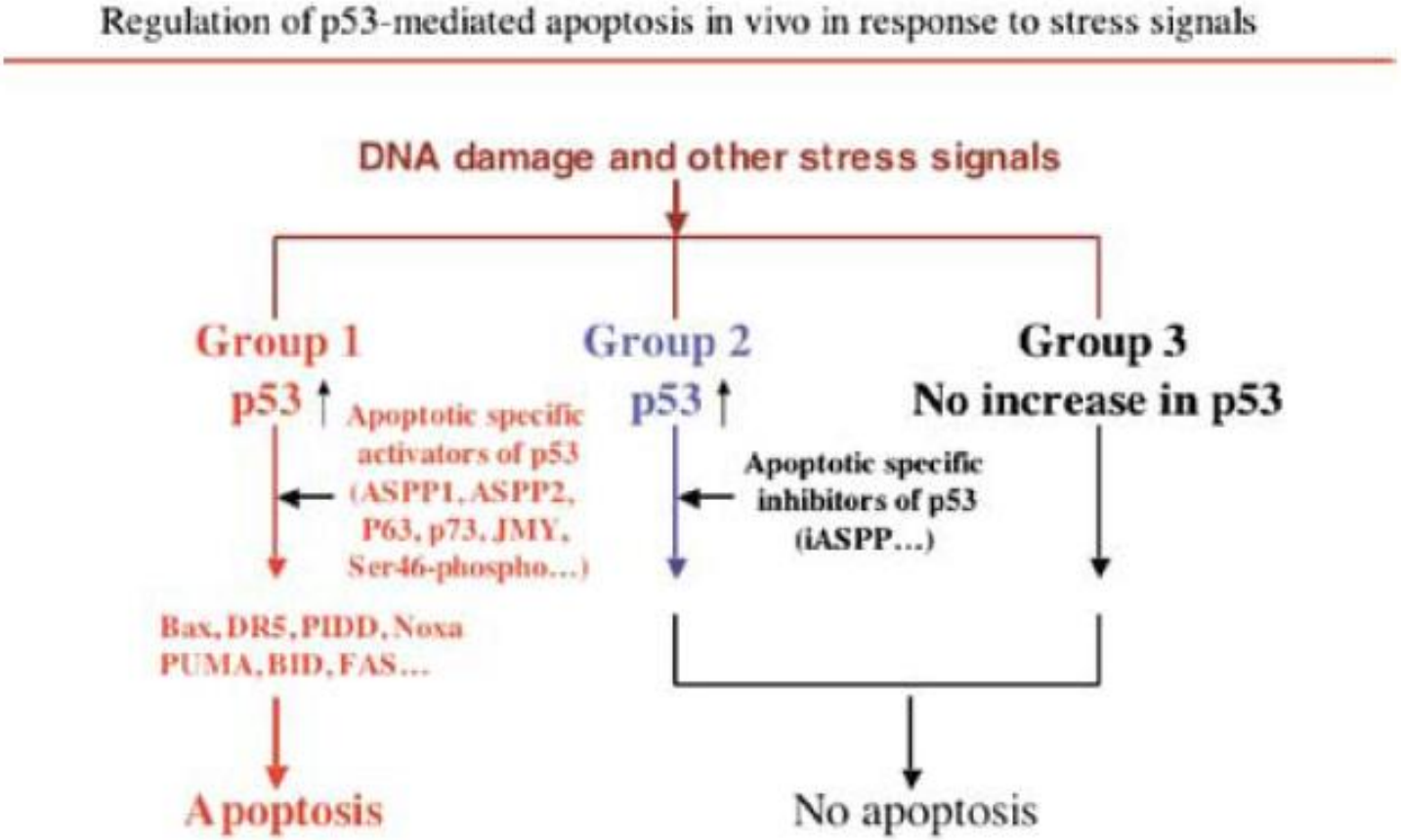


Figure 3 A diagram to illustrate how the apoptotic function of p53 can be regulated *in vivo* in a tissue-specific manner in response to various stress signals

P53 indukuje apoptózu vazbou na DNA nebo na mitochondrie

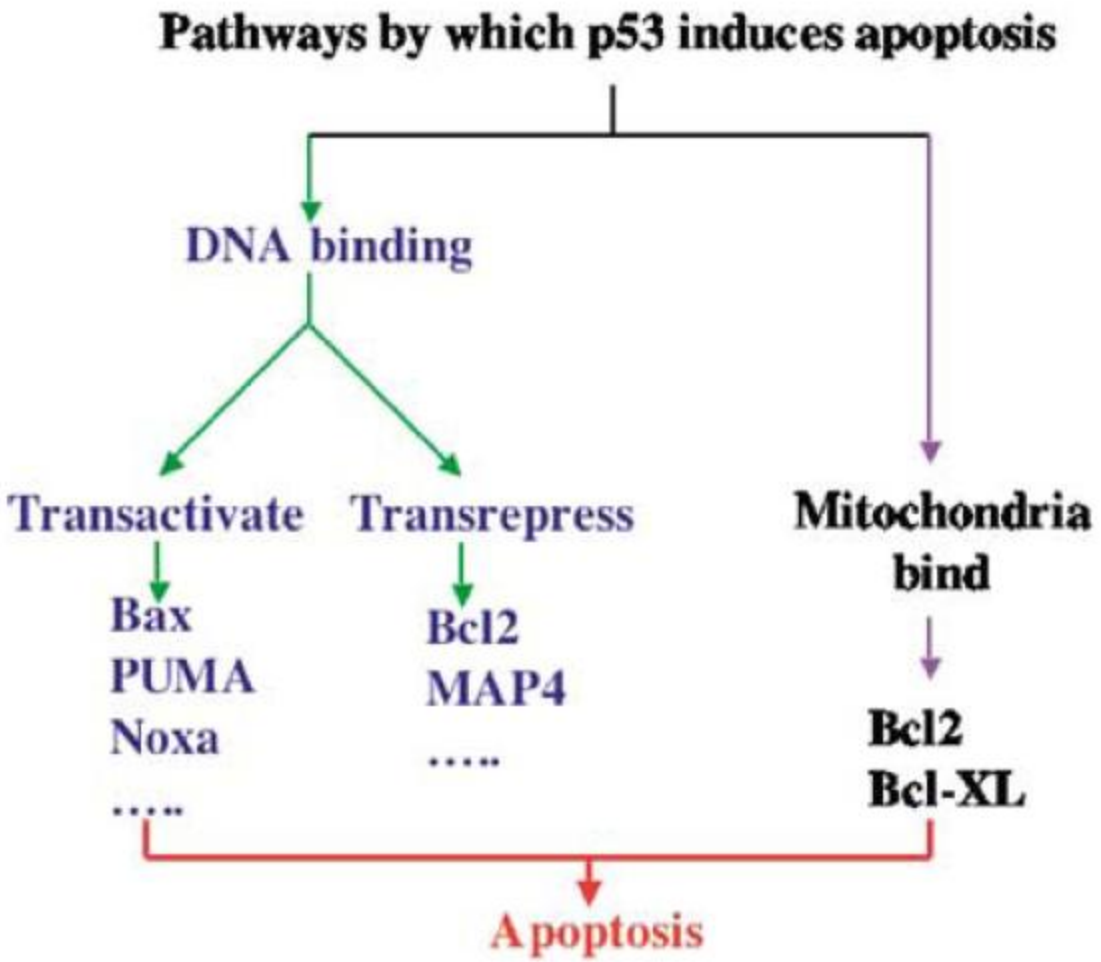


Figure 1 Pathways through which p53 induces apoptosis

Signální dráhy indukované p53 po apoptickém signálu

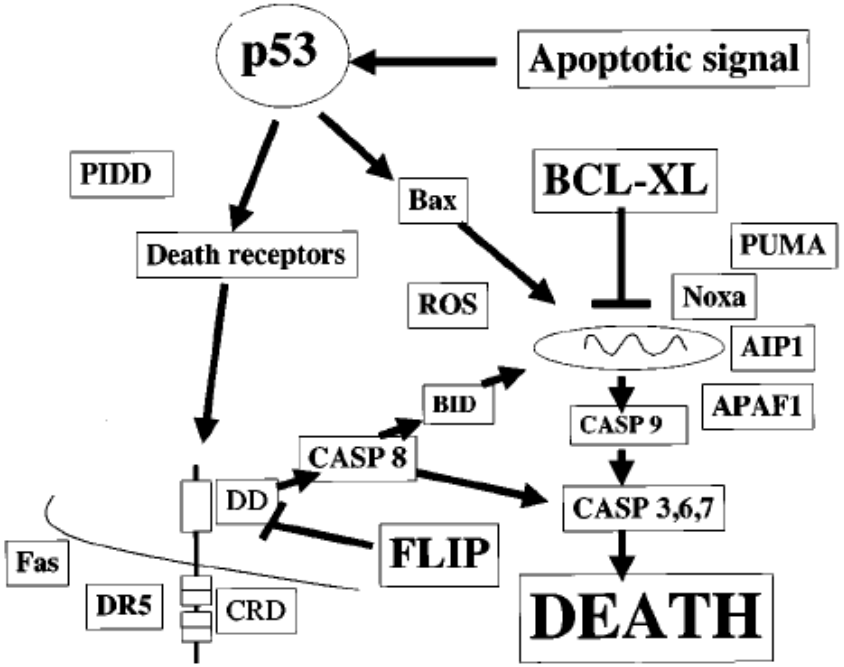
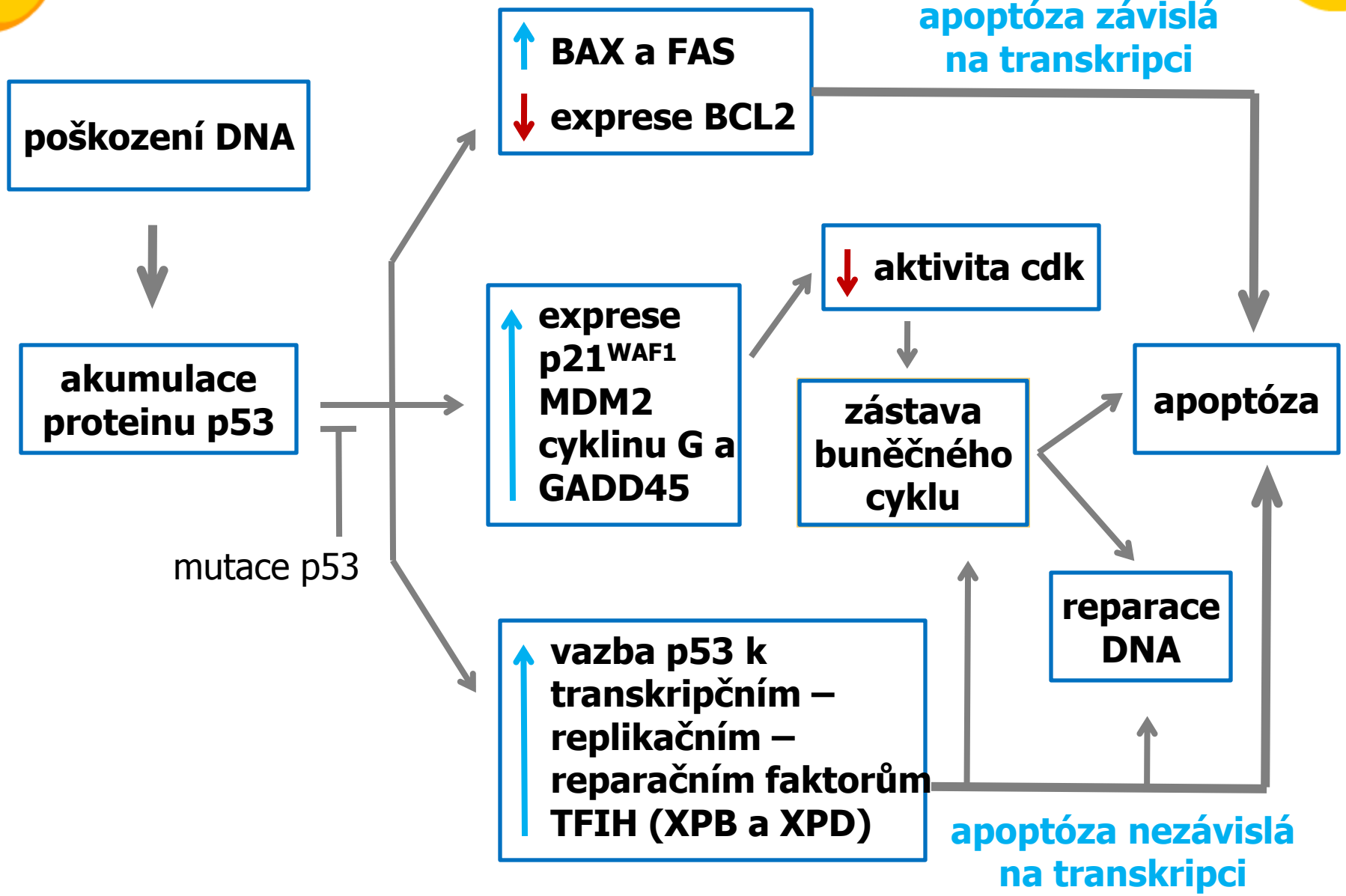
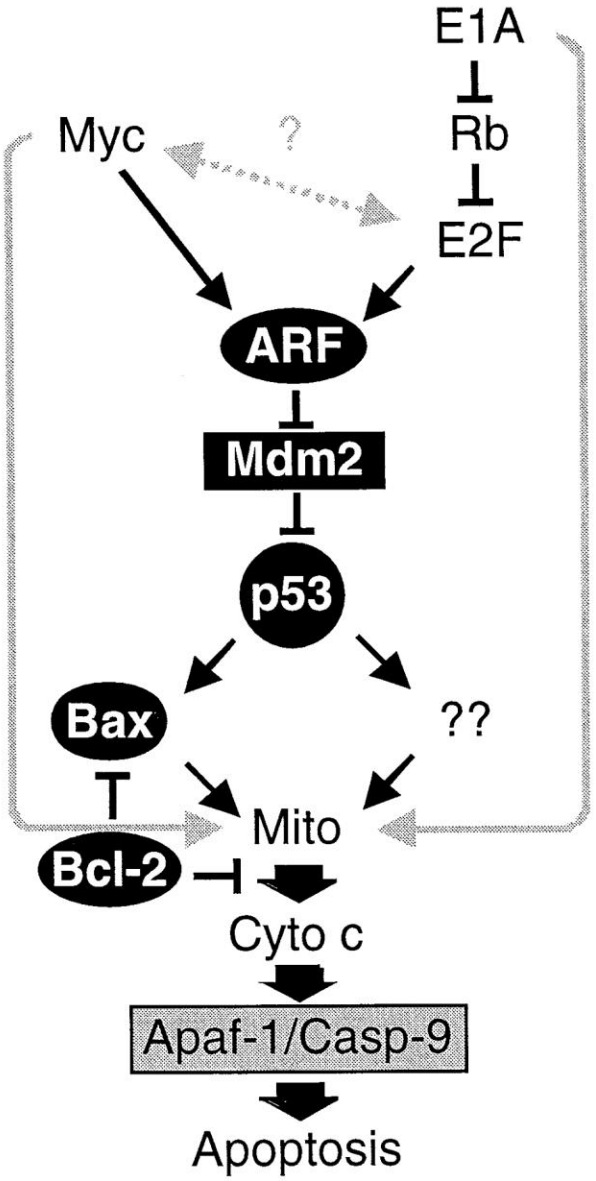


Figure 3 Pathways downstream of p53 that can cause cell death. In response to apoptotic signals, p53 protein is stabilized and activated resulting in transcriptional activation of multiple target genes that cause apoptosis of cells. These include death receptors such as Fas/APO1 or KILLER/DR5, or proteins that act more directly on caspase activation including Bax, Bak, Noxa, PUMA, AIP1, and APAF1. Death receptors contain extracellular cysteine-rich ligand-binding domains (CRD) and intracellular death domains (DD). Other targets including PIDD and PIG genes have been found to be activated by p53. PIG genes lead to the generation of reactive oxygen species (ROS) which are toxic to cells. The initiator caspases involved in p53 signaling of death include caspases 8 and 9, whereas the downstream executioner caspases include caspases 3, 6, and 7. Inhibitors of p53-dependent cell death include Bc1-X_L and FLIP, which act on cytoplasmic or mitochondrial pathways of death activation


poškození DNA
akumulace proteinu p53
mutace p53
↑ BAX a FAS
↓ exprese BCL2
apoptóza závislá na transkripci
↑ exprese p21WAF1 MDM2 cyklinu G a GADD45
↓ aktivita cdk
zástava buněčného cyklu
apoptóza
reparace DNA
↑ vazba p53 k transkripčním – replikačním – reparačním faktorům TFIH (XPB a XPD)
apoptóza nezávislá na transkripci

Apoptóza indukovaná onkogenem



Oncogenes such as *E1A* and c-*myc* induce apoptosis through p53-dependent and independent pathways, and both pathways may facilitate cytochrome c release from mitochondria. In any case, the Apaf-1/caspase-9 death effector complex appears important for oncogene-induced death. Current evidence has not ruled out the possibility that oncogenes and/or *p53* influence Apaf-1 and/or caspase-9 independent of cytochrome c, but this remains a possibility. Components of the oncogene-induced cell-death program that are mutated in human tumors are shown in black, candidate tumor suppressors are shown in gray.

Úloha survivinu v ochraně proti mitotické katastrofě

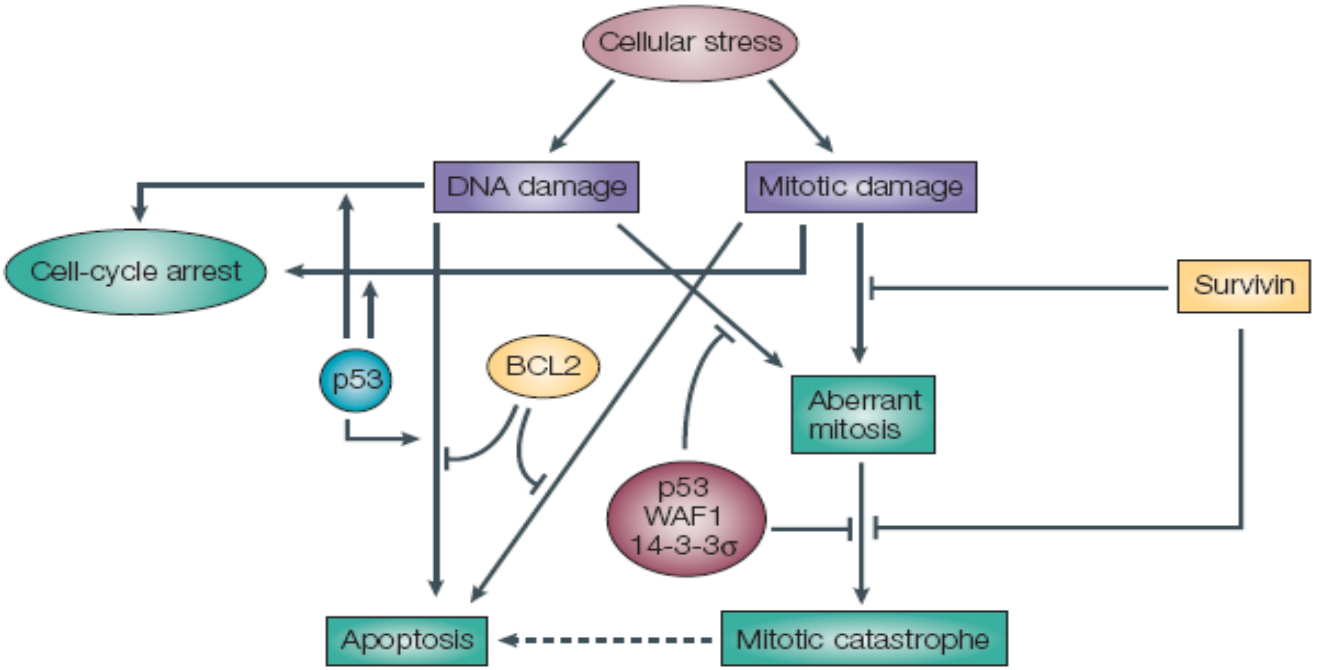


Figure 2 | **Model for survivin-mediated protection against mitotic catastrophe.** Cellular stresses induce both DNA damage and defects in the mitotic machinery (mitotic damage). DNA damage can lead to cell-cycle arrest, apoptosis or aberrant mitosis. Mitotic damage can lead to cell-cycle arrest, apoptosis or mitotic catastrophe through aberrant mitosis. In situations in which p53 activity has induced apoptosis, BCL2 expression can rescue cells from death. p53 — and its target genes that encode WAF1 and 14-3-3σ — also help to prevent aberrant mitosis and mitotic catastrophe. The normal function of survivin is to maintain the integrity of the mitotic spindle and promote mitotic progression. Loss of survivin induces cell-cycle arrest and cell death by mitotic catastrophe in a manner that is independent of both p53 and BCL2. However, loss of survivin also induces p53 and WAF1 expression, perhaps indirectly triggering p53-dependent apoptosis (dashed line).

Okada H. and Mak TW, Nature Rev Cancer 2004

Anti-apoptické signály z NF-kB

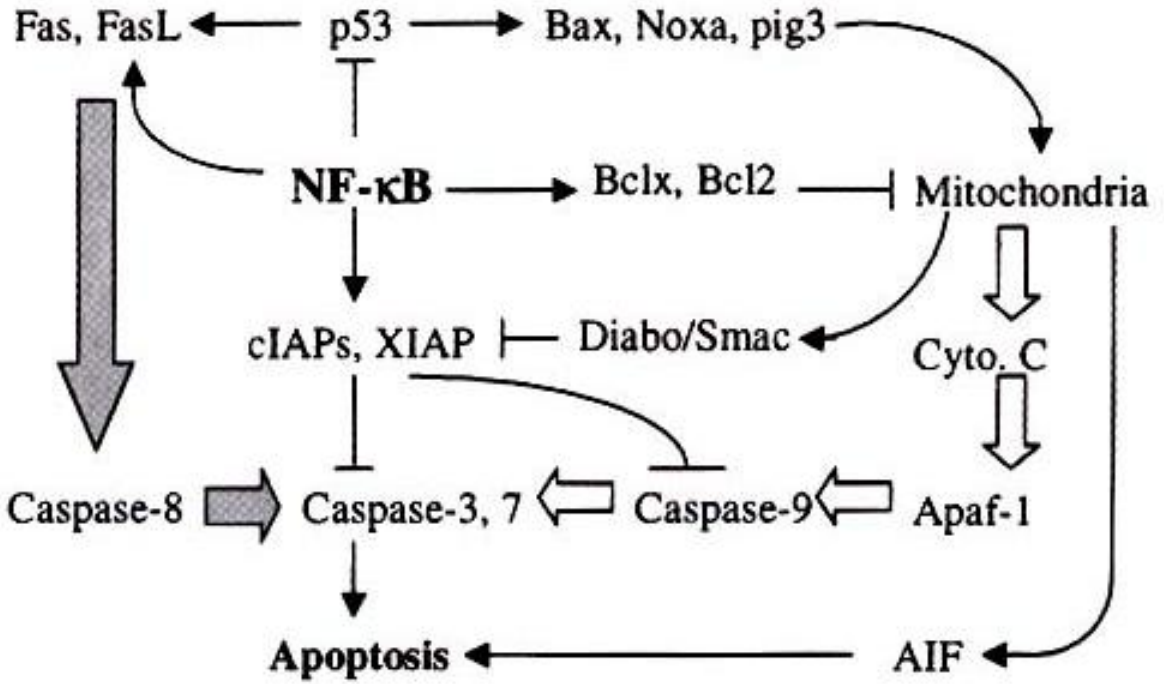
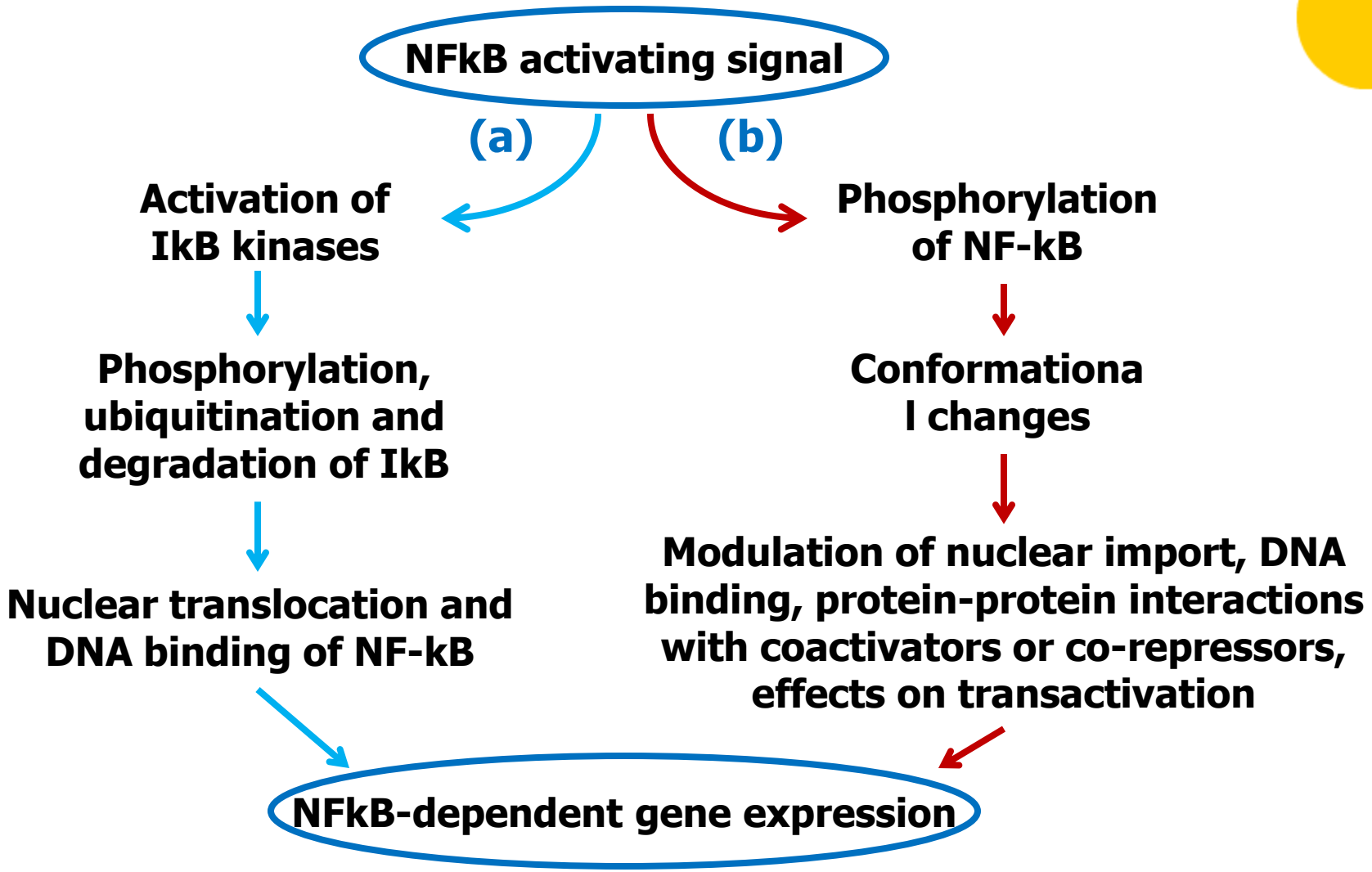


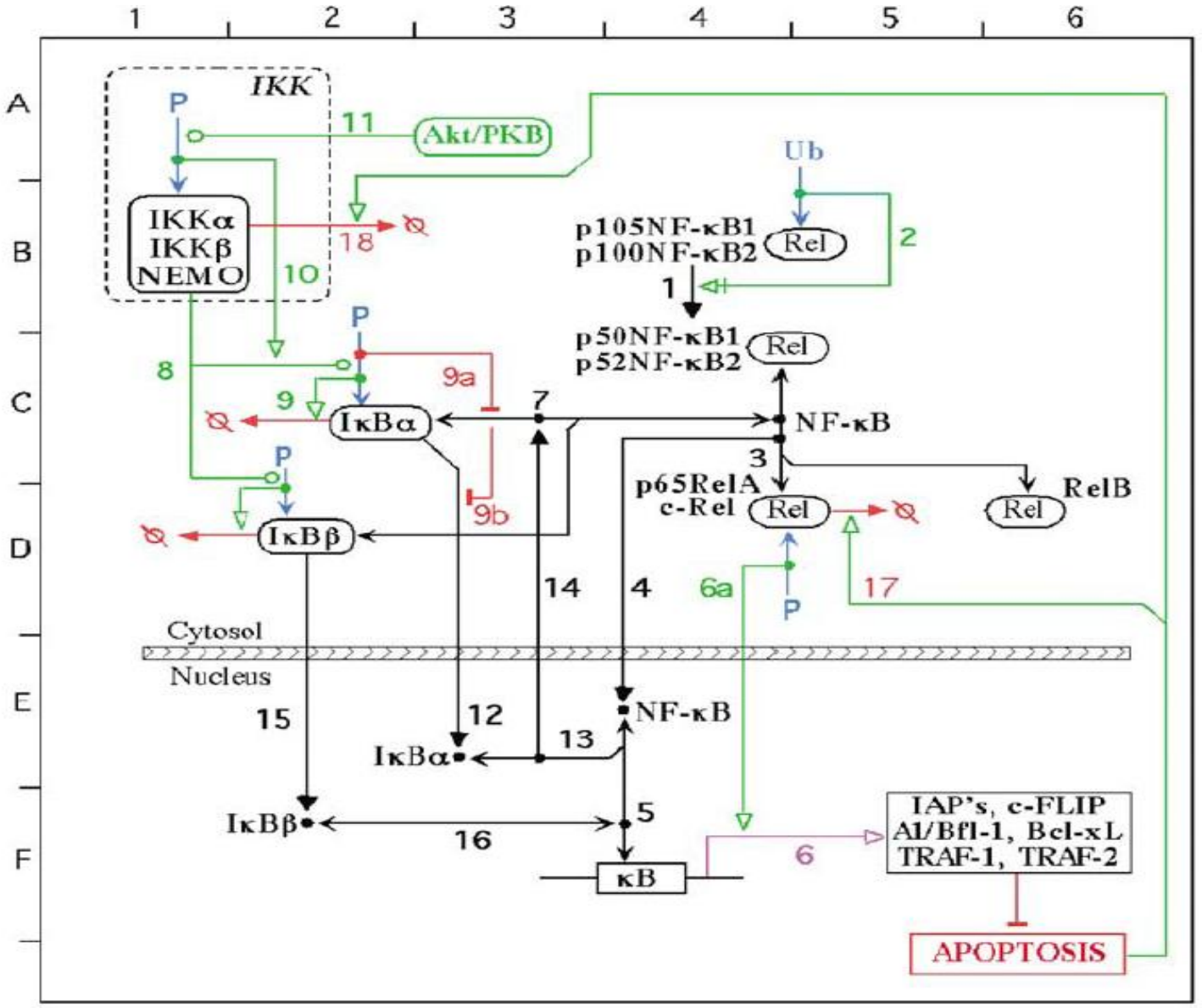
Figure 2. Possible targeting point of anti-apoptotic signals from NF-κB. Intrinsic (**open arrows**) and extrinsic (**filled arrows**) apoptosis pathways are depicted. The effector caspases, such as caspase-3 and caspase-7, are activated by upstream initiator caspases, caspase-8 and caspase-9. The initiator caspases themselves are activated by either ligands binding to the death receptor complex or cytochrome *c* released from damaged mitochondria. An anti-apoptotic effect of NF-κB is achieved through its up-regulation of IAPs that inhibits caspases and Bcl-xl that protects mitochondria from further damaging. →, activation; ⊣, inhibition.

Cíle antiapoptického působení NF-kB jsou součástí vnitřní i vnější dráhy apoptózy. NF-kB zvyšuje expresi IAPs, které inhibují kaspázy a Bcl-xl chránicího mitochondrie před poškozením.



Regulace aktivity NF-kB závislá a nezávislá na IkB. (a) NFkB je aktivován po aktivaci IkB kinázy (IKK). Tyto kinázy fosforylují IkB, což vede k jeho degradaci a jaderné translokaci uvolněného NF-kB. (b) Zároveň samotný NF-kB je fosforylován cytosolovými nebo jadernými protein kinázami, což zvyšuje účinnost genové exprese indukované NF-kB. IkB, inhibitor NF-kB; NF-kB, jaderný faktor kB.

Molekulární interakční mapa NFκB/I κB



Účinky nesteroidních antiflogistik (NSAIDs) na signální dráhy ovlivňující apoptózu

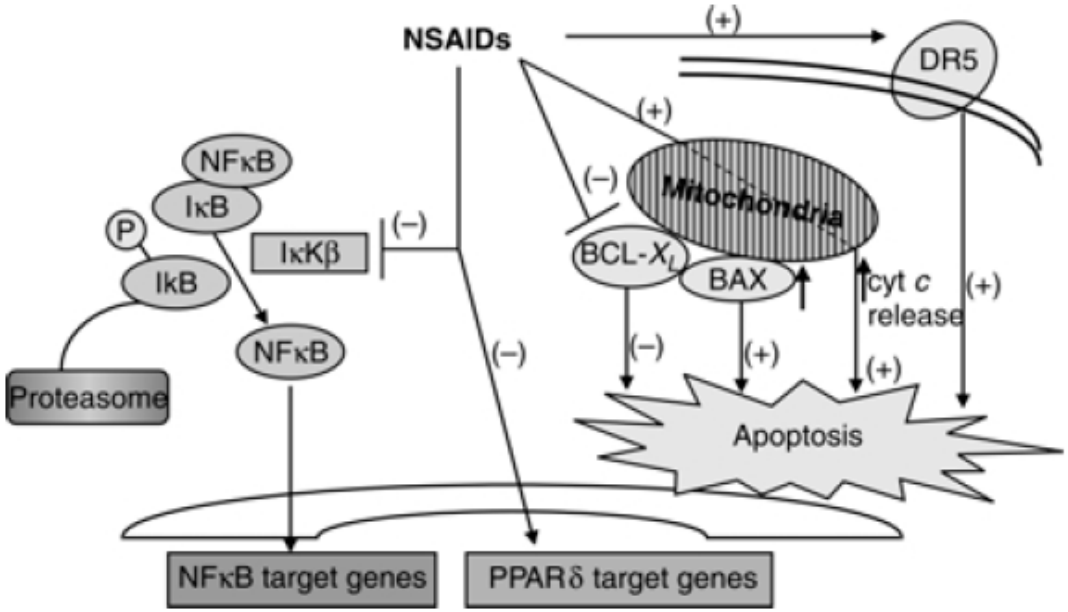


Figure 2. (1) NSAIDs inhibit activity of IκB kinase β (IκKβ) which inhibits NFκB signaling by blocking the degradation of IκB and thereby, preventing the translocation of NFκB to the nucleus. (2) NSAID (sulindac) can inhibit the DNA binding activity of PPARδ. (3) NSAIDs trigger both the mitochondrial and death receptor-mediated apoptotic pathways with resultant cytochrome c (cyt c) release and DR5 up-regulation, respectively. NSAIDs also inhibit the anti-apoptotic Bcl- X_L protein resulting in an increase in the ratio of pro-apoptotic BAX: Bcl-X_L

Využití apoptózy v protinádorové terapii

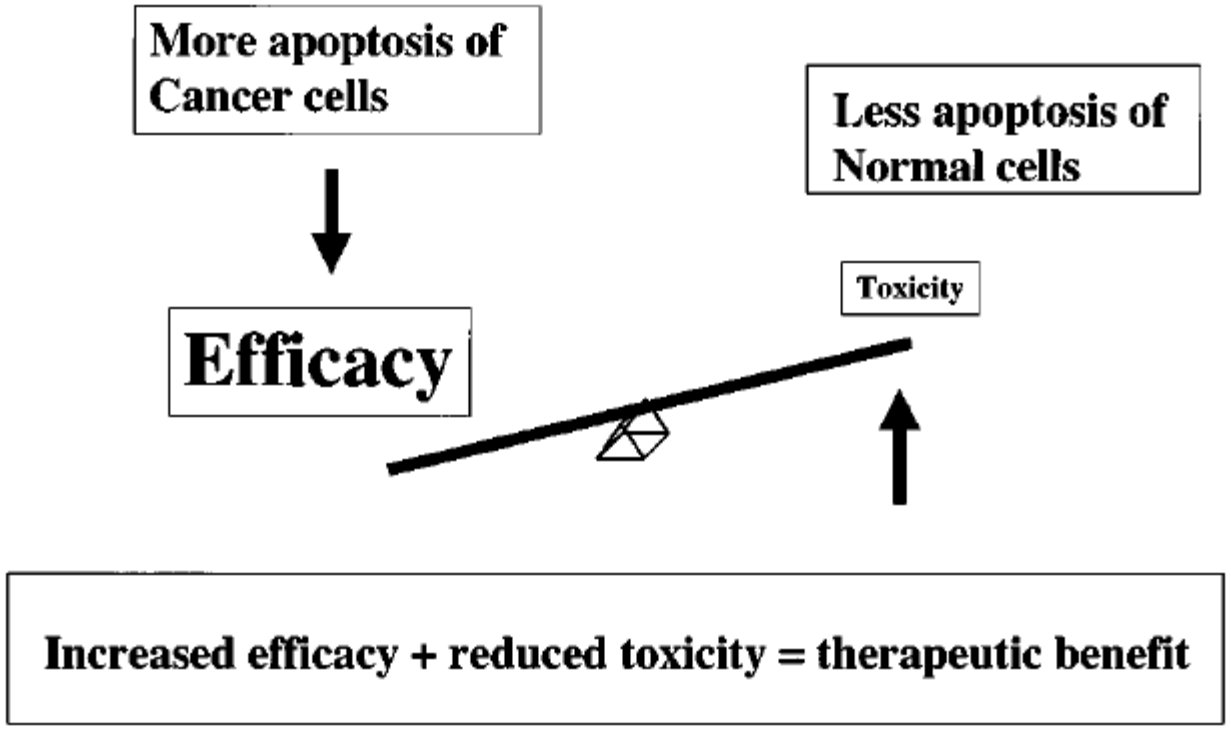


Figure 1 Desirable strategy to achieve therapeutic benefit in cancer therapy. Agents which effectively kill cancer cells are useful to the extent that toxicity to normal cells is tolerated. It is clear that therapeutic benefit can be achieved by increasing apoptosis of cancer cells as well as by lowering toxicity to normal cells upon exposure to anticancer therapy

Využití selektivní cytotoxicity endogenního induktoru apoptózy TRAIL v chemoterapii

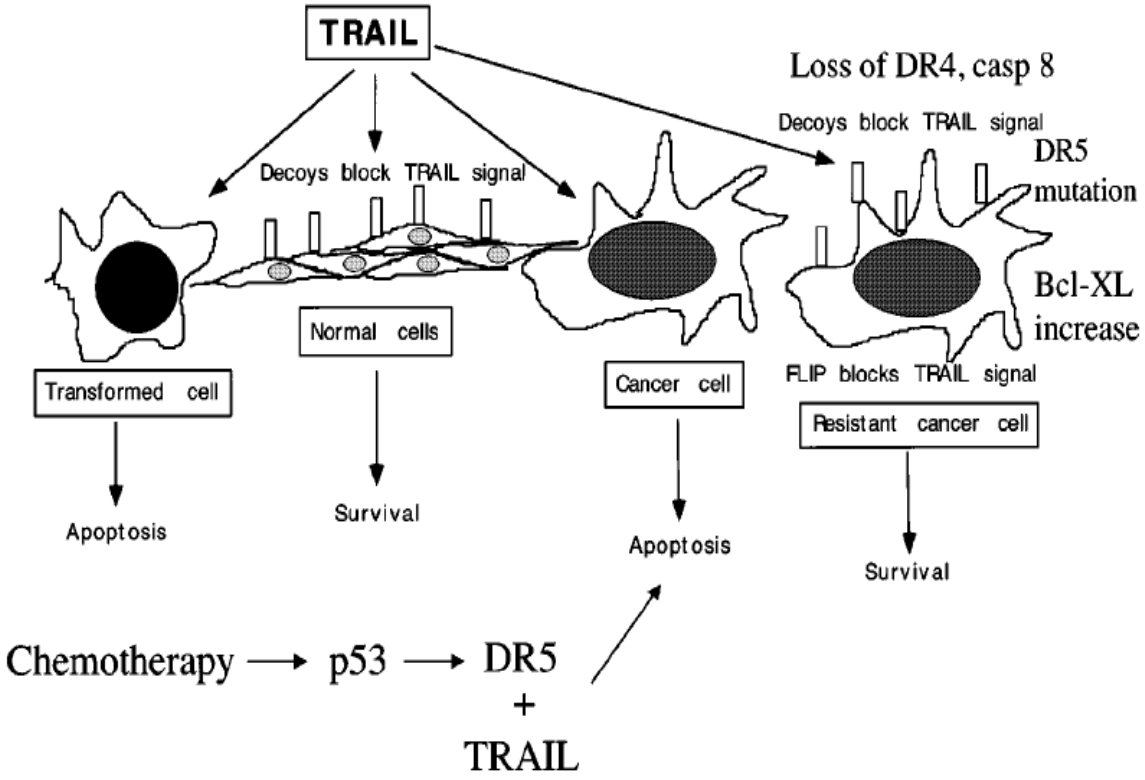


Figure 4 Sensitivity and resistance to TRAIL-induced cell death. The cytotoxic ligand TRAIL kills cancer and transformed cells but not most normal cells. Normal cells are believed to be protected from TRAIL because they tend to express higher levels of TRAIL decoy receptors (TRID or TRUNDD). Most cancer cells are sensitive to TRAIL, but there are multiple mechanisms of resistance that have been described. Cancer cells can become resistant to TRAIL if they lose expression of the proapoptotic TRAIL receptors DR4 or KILLER/DR5. This can occur through homozygous deletion of DR4 or mutations in DR5. Inactivating DR5 mutations have been described in head and neck, lung and breast cancers. TRAIL resistance may develop if cells overexpress either FLIP or Bc1-X_L. Neuroblastomas can become resistant to TRAIL through hypermethylation of caspase 8 which may be reversed by exposure to 5-aza-Cytidine. Chemotherapy or radiation can be combined with TRAIL to achieve synergistic cell killing, in part through p53-dependent upregulation of KILLER/DR5 expression

TRAIL indukuje apoptózu nádorových nikoli normálních buněk. Některé typy nádorových buněk jsou však k jeho účinkům rezistentní – kombinovaná terapie.

Indukce apoptózy dietetickými faktory

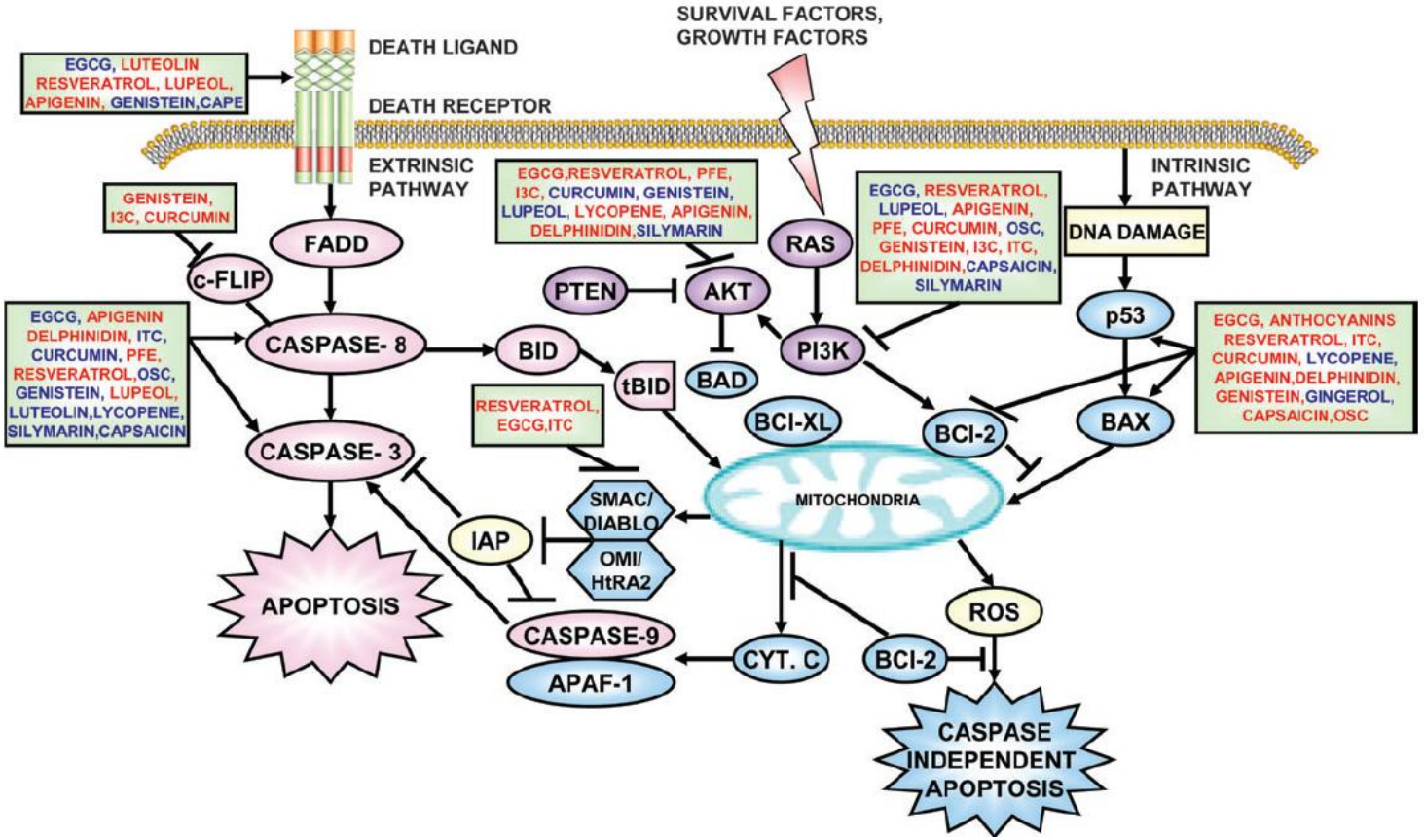
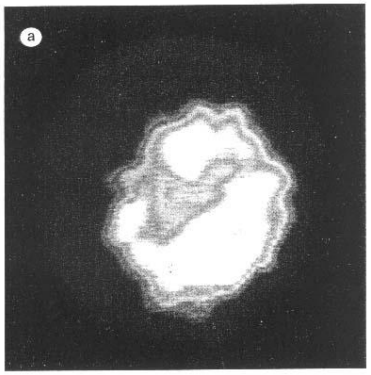


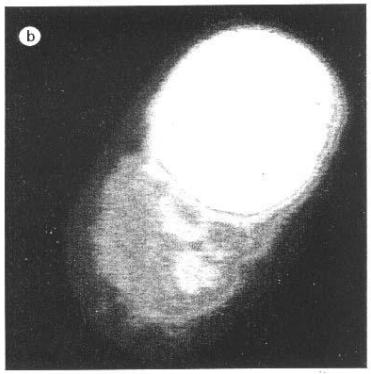
Fig. 1. Induction of apoptosis by dietary chemopreventive agents. The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor and TRAIL receptor) to activate membrane-proximal (activator) caspase-8 via the adaptor molecule FADD. This in turn cleaves and activates effector caspase-3. Dietary agents block the death receptor and also target the caspases blocking the caspase cascade. This pathway can be regulated by c-FLIP, which inhibits upstream activator caspases and IAPs, that affects both activator and effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins into the cytoplasm. Stress signals elicited by the dietary chemopreventive compounds regulate the proapoptotic proteins and antiapoptotic proteins, leading to the release of cytochrome *c* from the mitochondrial inner membrane. Cytochrome *c* forms an apoptosome with Apaf-1 and caspase-9, thereby initiating the apoptotic caspase cascade, whereas Smac/DIABLO and high-temperature requirement protein-A2 bind to and antagonize IAPs. The activated caspases catalyze the dissolution of intracellular structure that leads to apoptotic cell death. The Bcl-2 family proteins regulate apoptosis as they form complexes that enter the mitochondrial membrane, regulating the release of cytochrome *c* and other proteins. The activation of the caspase cascade occurs by the TNF family receptor and it also causes activation of Bid that activates mitochondria-mediated apoptosis. Bax is activated and releases cytochrome *c* and other mitochondrial proteins. Dietary agents can also block growth factor-mediated antiapoptotic signals through the direct inhibition of the binding of growth factors to the receptor or inhibition of the downstream phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Blue color of dietary chemopreventive agents denotes that both the *in vivo* and *in vitro* effects have been demonstrated and red color denotes that only *in vitro* effects have been demonstrated.

Khan N et al., Carcinogenesis 2007, 28:233

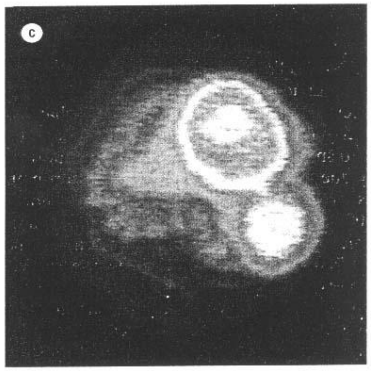
Stadia apoptózy (konfokální fluorescenční mikroskopie)



Viabilní buňka

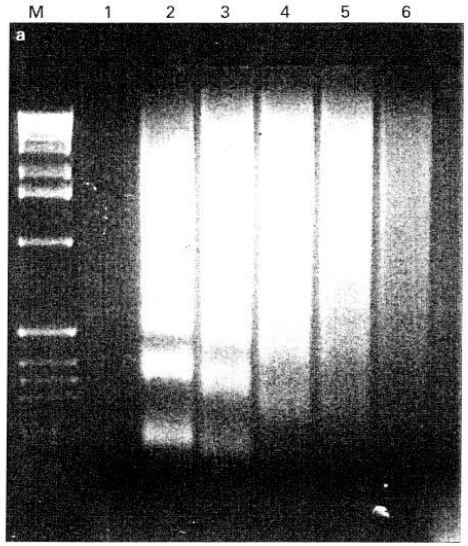


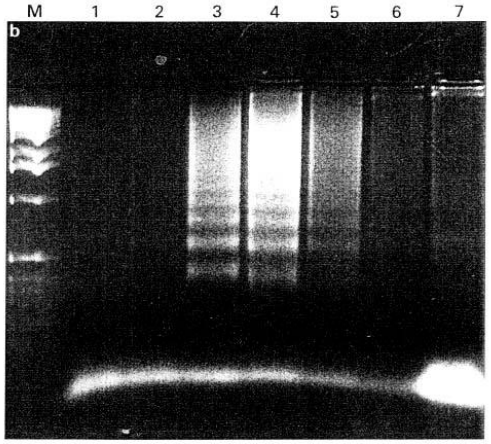
Rané stádium apoptózy
(zmenšování buňky, tvorba membránových měchýřků)



Střední stádium apoptózy
(kondenzace a fregmentace chromatimu, tvorba apoptotických tělísek)

Analýza fragmentace DNA během apoptózy





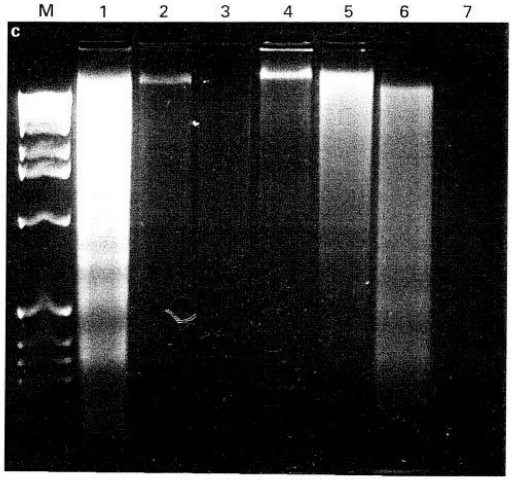
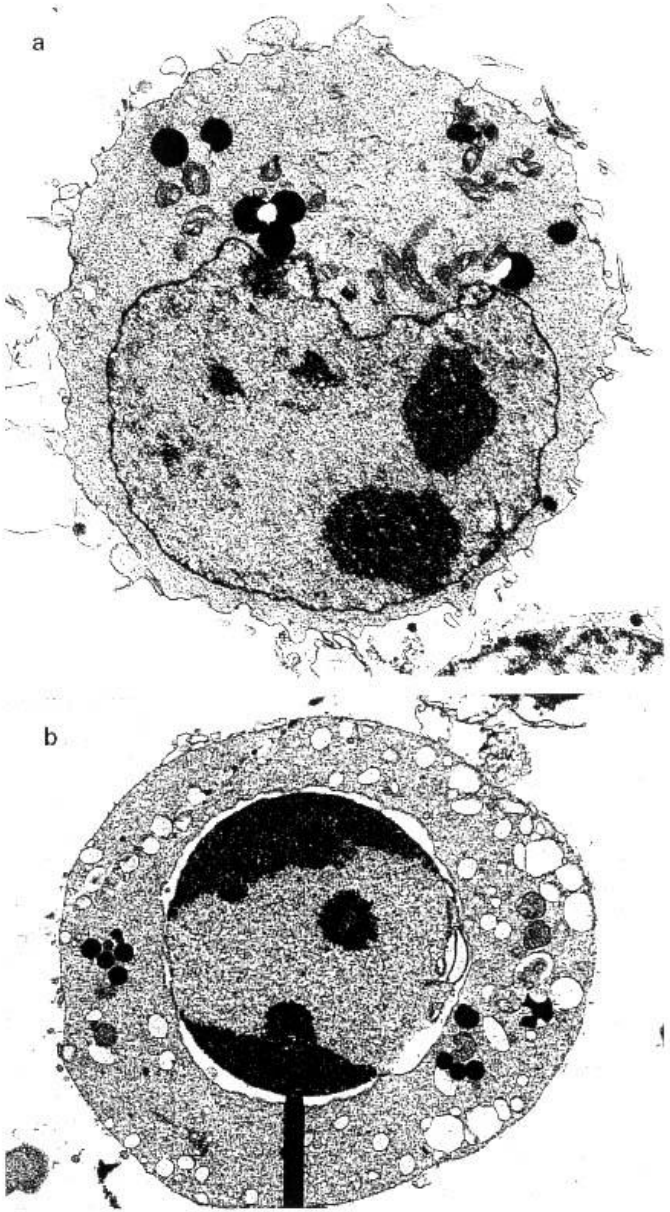


Fig. 4—Analysis of DNA fragmentation of apoptosis from three cell lines. (a) HL-60 cells, exposed to camptothecin (0·5 μM). Lanes: M = marker lane containing a 1 kb ladder of DNA fragments from 0·5 to 12·0 kb; 1 = control, time 0; 2 = + camptothecin, time 6 h; 3 = + camptothecin, time 12 h; 4 = + camptothecin, time 24 h; 5 = + camptothecin, time 30 h; 6 = + camptothecin, time 48 h. (b) HL-60 cells exposed to EPA (100 μM). Lanes: M = marker lane; 1 = control, time 6 h; 2 = + EPA, time 6 h; 3 = + EPA, time 12 h; 4 = + EPA, time 24 h; 5 = + EPA, time 30 h; 6 = + EPA, time 49 h; 7 = control, time 49 h. (c) c-*myc*-transfected fibroblasts after serum withdrawal (lanes 1–5) and Mia-Pa-Ca-2 cells exposed to 100 μM EPA (lanes 6 and 7). Lanes: M = marker lane as above; 1 = fibroblasts detaching between 23 and 35 h; 2 = fibroblasts attached at 48 h; 3 = fibroblasts detaching between 35 and 48 h; 4 = fibroblasts attached at 74 h; 5 = fibroblasts detaching between 48 and 74 h; 6 = Mia-Pa-Ca-2 cells detaching between 23 and 35 h showing a 'chromatin ladder' of apoptosis; 7 = Mia-Pa-Ca-2 cells detaching between 35 and 48 h

Tvorba žebříčku – „ladder"
po expozici leukemické linie HL-60 kampotecinem (a) nebo eikospentaenovou kyselinou - EPA (b).
c) Fibroblaty kultivované v médiu bez séra (sloupec 1-5) a pankreatické buňky po expozici EPA (sloupec 6,7).

Viabilní a apoptotické buňky v el. mikroskopu



Oxidativní stres jako mediátor apoptózy

Mnoho látek, které indukují apoptózu jsou buď oxidanty nebo stimulátory buněčného oxidativního metabolismu. Naopak řada inhibitorů apoptózy má antioxidační účinky.

Možné mechanismy:

- Bcl-2 protein (produkt bcl-2 onkogenu) - v mitochondriích, endopl. retikulu a jaderné membráně - regulace ROS
- Aktivace poly-ADP-riboso-transferázy a akumulace p53 - polymerizace ADP-ribózy s proteiny vyúsťuje v rychlou ztrátu zásoby NAD/NADH, kolaps zásob ATP a smrt buňky.
- Oxidace lipidů v bun. membránách - mediátory apoptózy HPETE (po působení TNFα)
- Aktivace genů odpovědných za apoptózu přes aktivaci specifických transkripčních faktorů jako je NFκB – rozporná úloha.
- AP-1, antioxidant-responsivní faktor může také přispívat k regulaci apoptózy.

Fyziologicky se ROS se tvoří v:

Peroxisomech - rozklad mastných kyselin (MK) - peroxid
Kataláza využívá peroxid v detoxifikačních reakcích

Mitochondriích - respirační cyklus a katabolismus MK. Mn superoxid dismutasa a další antioxidanta v mitochondriích udržují nízkou hladinu těchto ROS. Byla prokázána silně inverzní korelace mezi produkcí ROS mitochondriemi a délkou existence savčího druhu.

Mikrosomální systém transportu elektronů (cytochrome P450) - vyžaduje elektrony z NADPH k produkci částečně redukovaných kyslíkových druhů. ROS vznikají jen za přítomnosti selektovaných xenobiotik - superoxidový radikál - konverze na reaktivnější hydroxylový radikál

Mimobuněčné děje - oxidativní vzplanutí aktivovaných makrofágů - NADPH-oxidáza -superoxid.

Antioxidační obranný systém:

- neenzymatický: molekuly jako vit E, vit C a glutation působící přímo na ROS

- enzymatický: superoxid dismutáza (SOD), kataláza (CAT), GSH peroxidasa (GSH-Px) a GSH S transferasa (GST). Mohou buď přímo odstraňovat ROS nebo působit recyklaci neenzymatických molekul.

Zdroje a mediátory oxidativního stresu

Table 1. *Mediators of Oxidative Stress*

- **Reactive Oxygen Species**
  - **Free radicals**
    - **Hydroxyl radical (HO·)**
    - **Superoxide radical ($O_2^{\cdot -}$)**
  - **Nonradicals**
    - **Hydrogen peroxide (H_2O_2)**
    - **Singlet oxygen ($^1O_2$)**
  - **Lipid Peroxidation Products**
    - **Peroxyl radical (ROO·)**
    - **Alkoxyl radical (RO·)**
  - **Secondary Products**
    - **Malondialdehyde**
    - **4-Hydroxyalkenals**

Reaktivní kyslíkové metabolity (ROS)

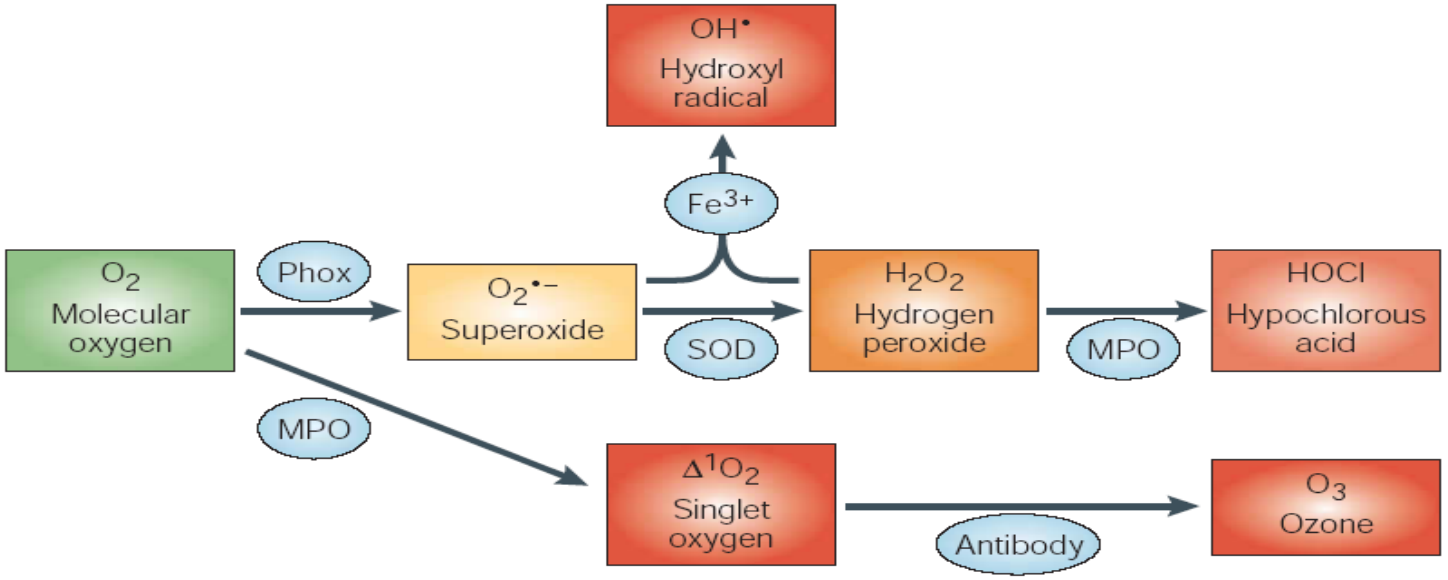


Figure 1 | **Reactive oxygen species.** Superoxide is generated from various sources, which include the NADPH oxidase (NOX) enzymes (such as the phagocyte NOX, Phox). Two molecules of superoxide can react to generate hydrogen peroxide (H_2O_2) in a reaction known as dismutation, which is accelerated by the enzyme superoxide dismutase (SOD). In the presence of iron, superoxide and H_2O_2 react to generate hydroxyl radicals. In addition to superoxide, H_2O_2 and hydroxyl radicals, other reactive oxygen species (ROS) occur in biological systems. In inflamed areas, these include hypochlorous acid (HOCl), formed in neutrophils from H_2O_2 and chloride by the phagocyte enzyme myeloperoxidase (MPO); singlet oxygen, which might be formed from oxygen in areas of inflammation through the action of Phox and MPO-catalysed oxidation of halide ions[64]; and ozone, which can be generated from singlet oxygen by antibody molecules[65,66]. The last reaction is likely to be important in inflamed areas in which antibodies bound to microorganisms are exposed to ROS produced by phagocytes. The colour coding indicates the reactivity of individual molecules (green, relatively unreactive; yellow, limited reactivity; orange, moderate reactivity; red, high reactivity and non-specificity). For further details see BOX 1.

Transmembránová topologie a doménová struktura NADPH oxidáz (NOX) a duálních oxidáz (DUOX)

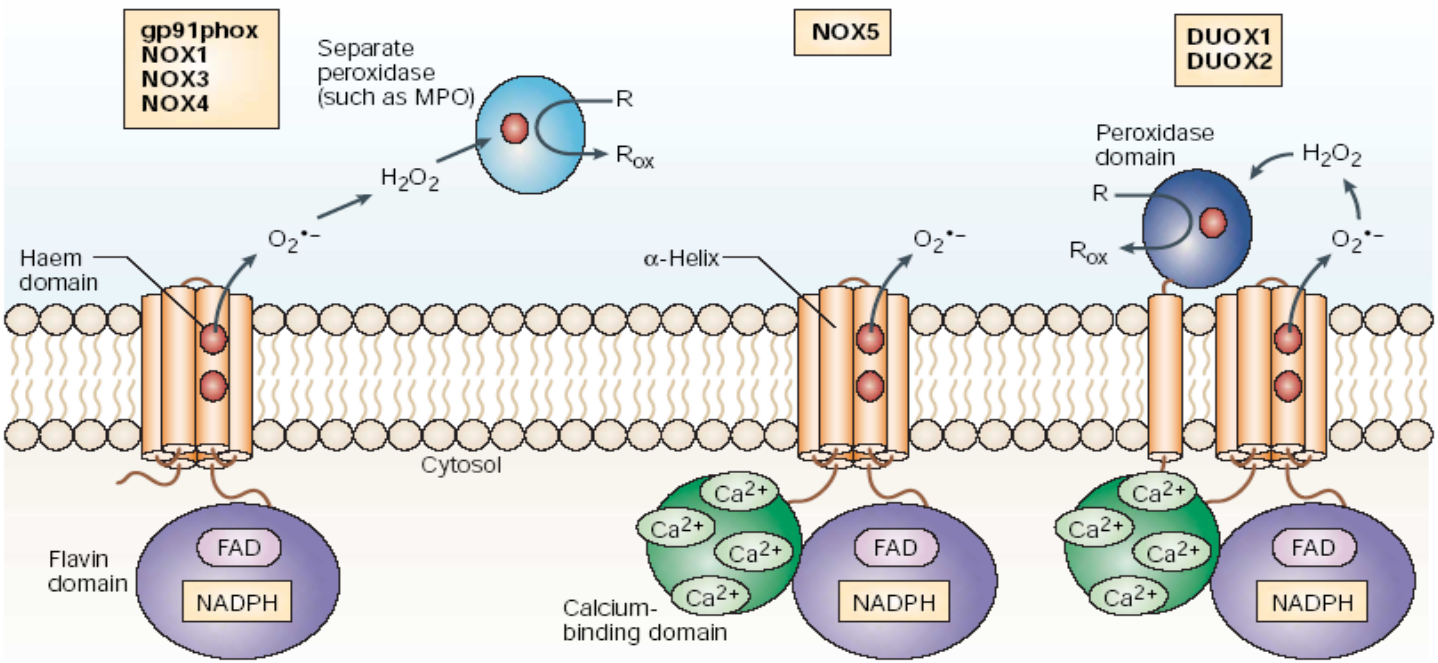


Figure 2 | **Transmembrane topology and domain structure of NOX and DUOX enzymes.** NADPH oxidase 1 (NOX1), NOX3 and NOX4 are similar in size and domain structure to the well-studied gp91phox, also known as NOX2. They contain an amino-terminal hydrophobic domain that is predicted to form six transmembrane α-helices. This region contains five conserved histidine residues, four of which provide binding sites for two haems. Haem is an iron-containing prosthetic group found in enzymes, electron transfer proteins and oxygen-binding pigments such as haemoglobin. The iron in haems is capable of undergoing reduction and re-oxidation, thereby functioning as an electron carrier. The two haems are located approximately within the two leaflets of the membrane bilayer, and together provide a channel for electrons to pass across the membrane. The carboxy-terminal portion of the molecule folds into an independent cytoplasmic domain that contains binding sites for the co-enzymes flavin adenine dinucleotide (FAD) and NADPH. The NOX enzymes catalyse the NADPH-dependent reduction of oxygen to form superoxide, which can react with itself to form hydrogen peroxide (H_2O_2). For gp91phox, the H_2O_2 serves as a substrate for myeloperoxidase (MPO), but it is not known whether other NOX enzymes provide H_2O_2 for separate peroxidase enzymes. NOX5 contains the same gp91phox-like catalytic core, plus an amino-terminal calcium-binding domain. The dual oxidase (DUOX) enzymes build on the NOX5 structure by adding at the amino terminus an extra transmembrane α-helix followed by a domain that is homologous to peroxidases such as MPO. This peroxidase-homology domain is predicted to be localized on the outside of the membrane, where it can use ROS generated by the catalytic core to generate more powerful oxidant species that then oxidize extracellular substrates (R).

Hlavní komponenty antioxidační sítě v buňce

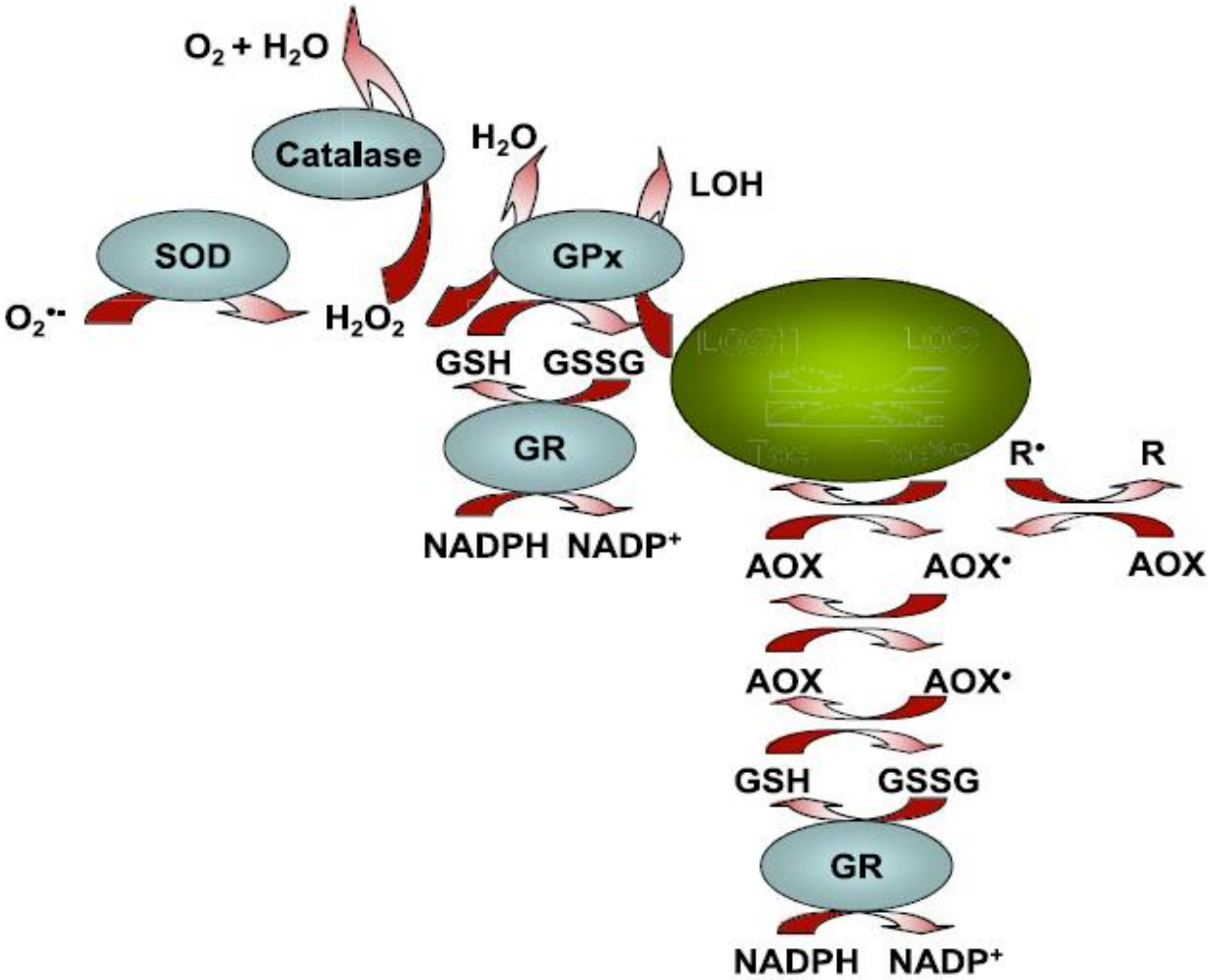


Fig. 1. Schematic representation of the major players of the cellular anti-oxidant network. The superoxide anion ($O_2^{\bullet-}$) is dismutated by superoxide dismutase (SOD), present in mitochondria and the cytosol. The produced H_2O_2, which could give rise to the formation of the extremely noxious hydroxylradical, can be neutralized by catalase (in the peroxysomes) and by the cytosolic and mitochondrial glutathione peroxidase (GPx). The latter enzyme removes H_2O_2 by oxidizing glutathione (GSH) to GSSG, which is subsequently reduced to its original from by Glutathion Reductase (GR), at the expense of NADPH. A second form of GPx can reduce more complex hydroperoxides, such as lipid-hydroperoxides (LOOH). Low molecular weight antioxidants or scavengers, such as tocopherol, ascorbate and glutathione, can neutralize radicals (for instance the peroxylradical ($LOO^{\bullet}$) and other radicals ($R^{\bullet}$)) and are often subsequently regenerated by one or more other antioxidants (AOX and GSH). Tocopherol (Toc) is an AOX that resides in cellular membranes (green circle), whereas other AOXs, such as ascorbate and GSH, are located in the cytosol. For an extensive review of the cellular antioxidant network one is referred to Halliwell and Gutteridge, 1999.

Boonstra and Post, Gene 2004

Antioxidační enzymy jsou využívány u onemocnění s nadprodukcí ROS

The use of anti-oxidant enzymes in reactive species overload diseases

	Glutathione	Catalase	Superoxide dismutase
IBD	Glutathione and its precursor inhibits[a]	Inhibits[a]	Inhibits[a]
Hepatitis	N-acetylcysteine does not appear to have an effect	Not examined	Not examined[b]
Gastritis	Not examined	Not examined	Not examined
Pancreatitis	Glutathione and N-acetylcysteine inhibit[a]	Inhibits[a]	Inhibits[a]
Esophagitis	Not examined	Inhibits[a]	Inhibits[a]
Prostatitis	Not examined	Not examined	Not examined
Cystitis	Not examined	Not examined	Not examined

[a] Animal studies.

[b] Although a superoxide dismutase mimetic has been shown to inhibit FAS-induced liver failure in mice [132].

Hofseth LJ and Ying L, BBA 2006, 1765:74

Pro- a protizánětlivé cytokiny u onemocnění s nadprodukcí ROS

a) Využívají se rekombinantní antagonisté nebo protilátky blokující aktivitu specifického cytokinu

b) Data ze studií na zvířatech (colitis – ulcerativní kolitida nebo Crohnova nemoc)

c) IFNgamma může být využit jako antivirová látka proti virové hepatitidě B v kombinaci s dalšími IFN

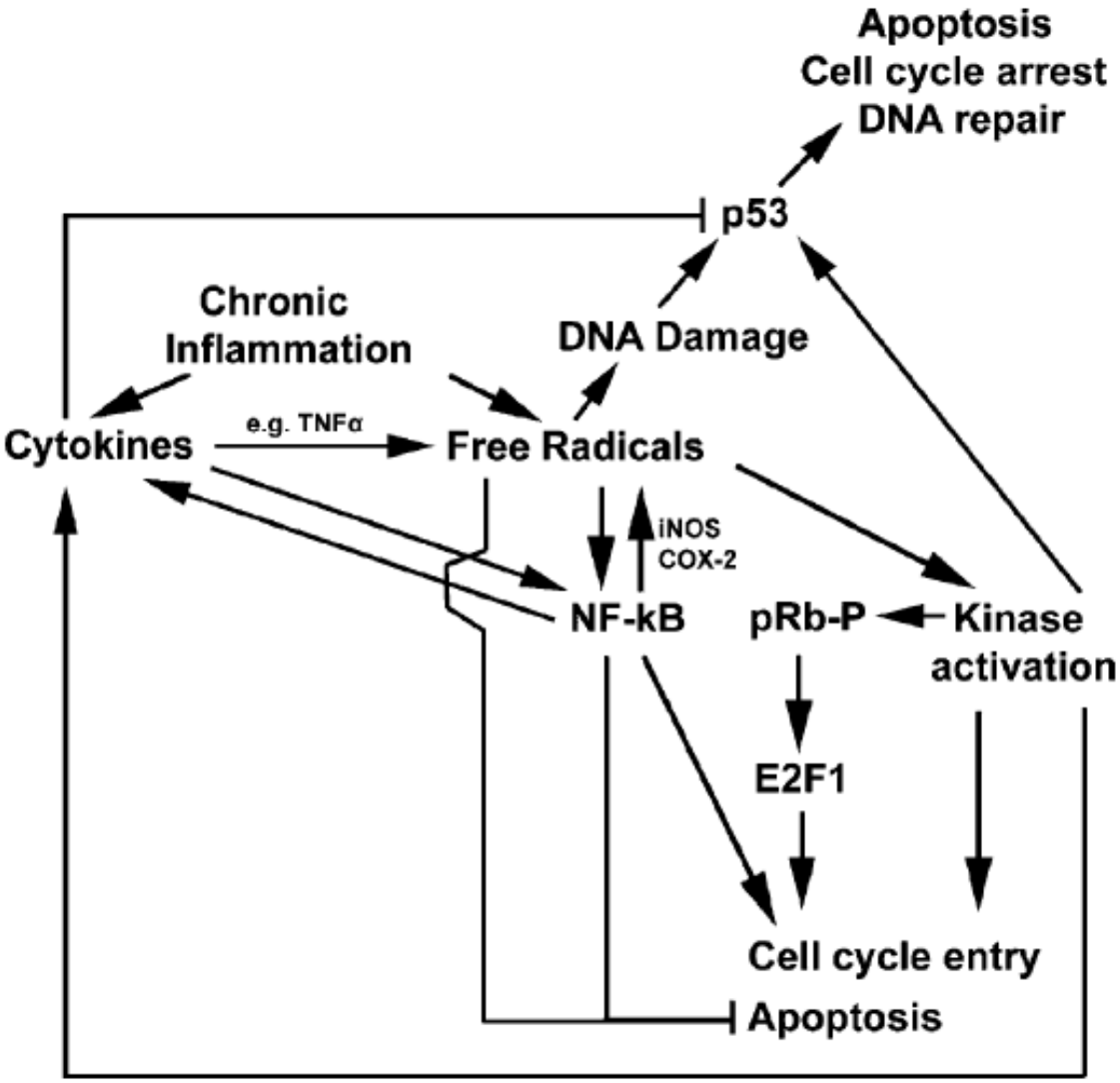
d) Rekombinantní cytokiny nebo metody stimulace protizánětlivých cytokinů

Cytokines in the treatment of reactive species overload diseases

Cytokine	Disease	Examples of cancer pathways affected
Pro-inflammatory[a]		
IL-1	Colitis[b], Pancreatitis, Hepatitis, Cystitis	Induces nitric oxide synthase (iNOS); induces COX-2; inhibits DNA repair
IL-6	Colitis[b] (Crohn's in humans)	Antagonize p53; pRb hyperphosphorylation; induces Bcl-2 and Bcl-XL; inhibits apoptosis
IL-7	Colitis[b]	Induces myc transcription
IL-12	Colitis[b] (Crohn's in humans), Hepatitis	Inhibits apoptosis and induces nucleotide excision repair; induces iNOS and NF-κB
IL-16	Colitis[b] (Crohn's in Humans)	Induces kinase signaling cascades
IL-18	Colitis[b], Hepatitis	Induces NF-κB
TNF-α	Colitis[b] (Crohn's in humans), Hepatitis, Cystitis	Induces NF-κB, iNOS and COX-2
IFN-γ	Hepatitis[c]	Activates fas-mediated apoptosis; induces iNOS and COX-2; induces DNA repair
MIF	Colitis[b]	Antagonizes p53
Chemokines		
CCR1/5	Colitis[b]	Affects proliferation in a p53-dependent manner
IP-10	Hepatitis	Unknown in hepatocytes
Mig	Hepatitis	Unknown in hepatocytes
Anti-inflammatory[d]		
IL-2	Colitis[b]	Induces fas-mediated apoptosis
IL-4	Gastritis	Induces pRb hypophosphorylation; decreases cyclin D1 and myc
IL-10	Colitis[b] (Crohn's in humans), Hepatitis, Pancreatitis, Gastritis	Inhibits proliferation
IL-11	Colitis[b] (Crohn's in humans)	Promotes pRb dephosphorylation
IFN-α	Hepatitis, Colitis[b] (UC in humans) Pancreatitis	Activates p53, Inhibits proteasomal degradation of survivin; promotes TRAIL and fas-mediated apoptosis; inhibits proliferation
IFN-β	Colitis[b] (UC in humans), Hepatitis	Induces apoptosis

Hofseth LJ and Ying L, BBA 2006, 1765:74

Některé klíčové dráhy u nemocí s vysokým oxidativním stresem vedoucí ke karcinogenezi



Hofseth LJ and Ying L, BBA 2006, 1765:74

Využití protizánětlivých látek s mnoha cíli u onemocnění s nadprodukcí ROS

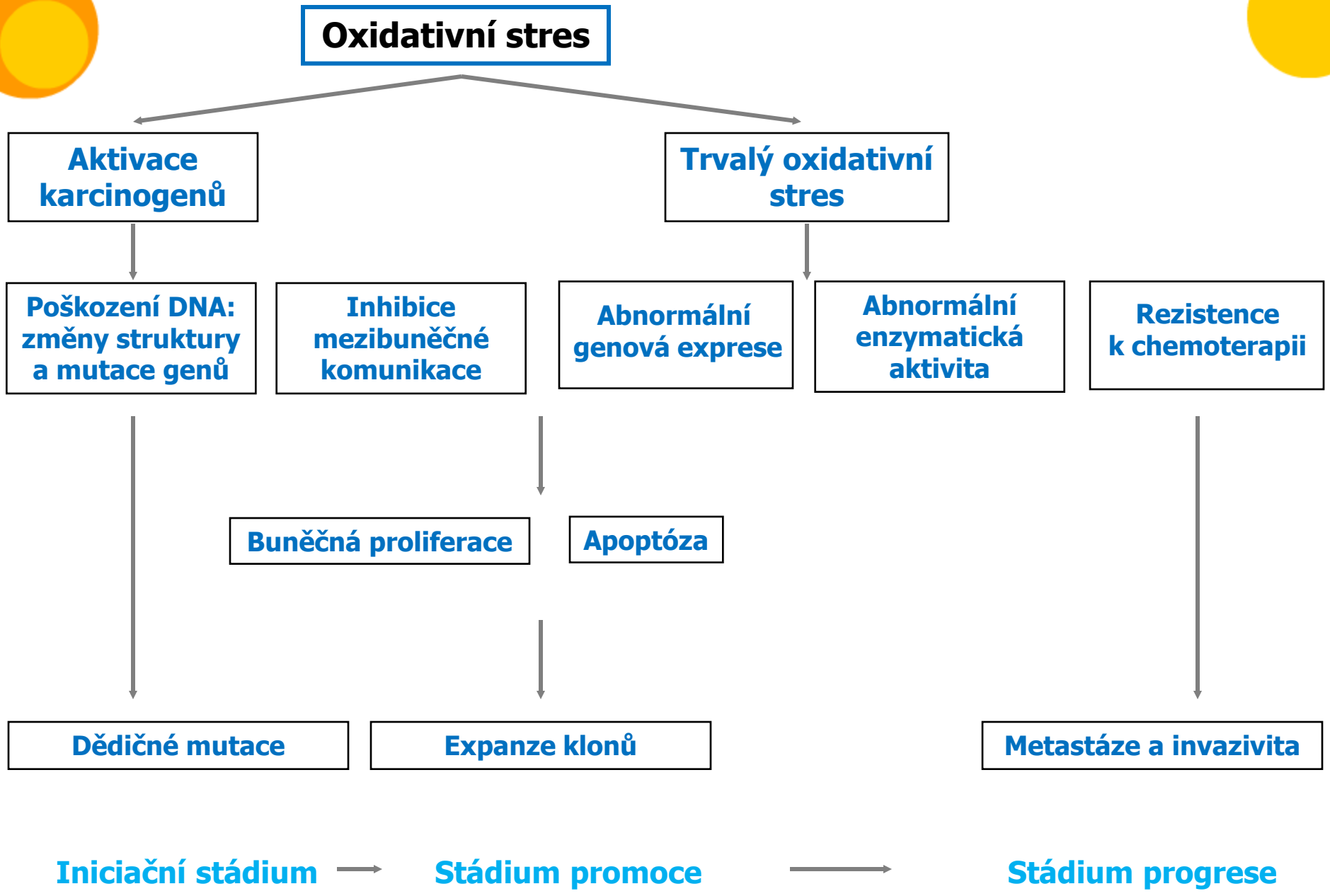
The use of anti-inflammatory agents with multiple targets in reactive species overload diseases

	NSAIDs	Vitamins	Trace minerals
IBD	5-ASA inhibits[a] Other NSAIDs tested may exacerbate[c]	Vit. C and E inhibit Crohn's oxidative stress[b] Folate may inhibit[c]	Selenium[c] and zinc may inhibit; iron may exacerbate[c]
Hepatitis	May exacerbate[d]	Vit. E inhibits viral Hepatitis[b]	Zinc inhibits[b,e] Iron and copper exacerbate[b,e]
Gastritis	Contradicting results	β-carotene and vit. C[b]	Selenium may inhibit[c]
Pancreatitis	May exacerbate[c]	Vit. C[b] and Combination treatment[b,f]	Selenium inhibits[a,h] Calcium may exacerbate[c]
Esophagitis	May inhibit[c]	Combination treatment[b,g]	Selenium may inhibit[c]
Prostatitis	May inhibit	Not examined	Zinc may inhibit[c]
Cystitis	May inhibit	Vitamin A[c]	Not examined

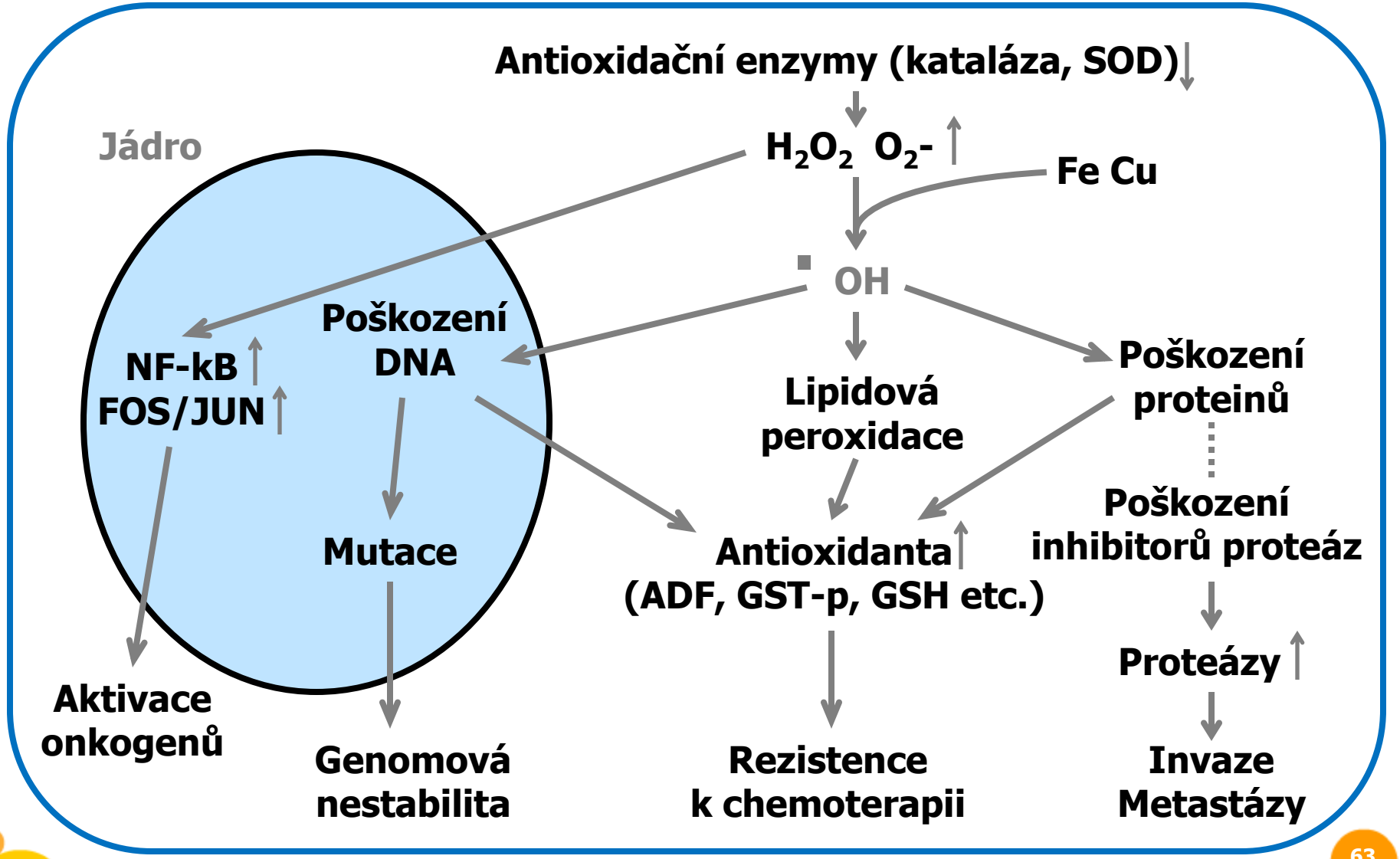
[a] Evidence is stronger for ulcerative colitis than Crohn's disease [6].
[b] Randomized prospective trial has been done (see text for citation).
[c] Data comes only from animal studies or retrospective human trials; no prospective human trial has been done for ulcerative colitis or Crohn's.
[d] Except when used in conjunction with IFN-α.
[e] Enhances the response to interferon-α therapy.
[f] Combinations included: selenium, β-carotene, and vitamins C and E [45], methionine, vitamin C and selenium [28]; selenium, β-carotene, vitamin C, vitamin E and methionine [29] or sulphadenosyl-methionine, Vitamin C, Vitamin E and Vitamin A [26].
[g] β-carotene, vitamin E and retinol [36].
[h] In combination with other antioxidants.

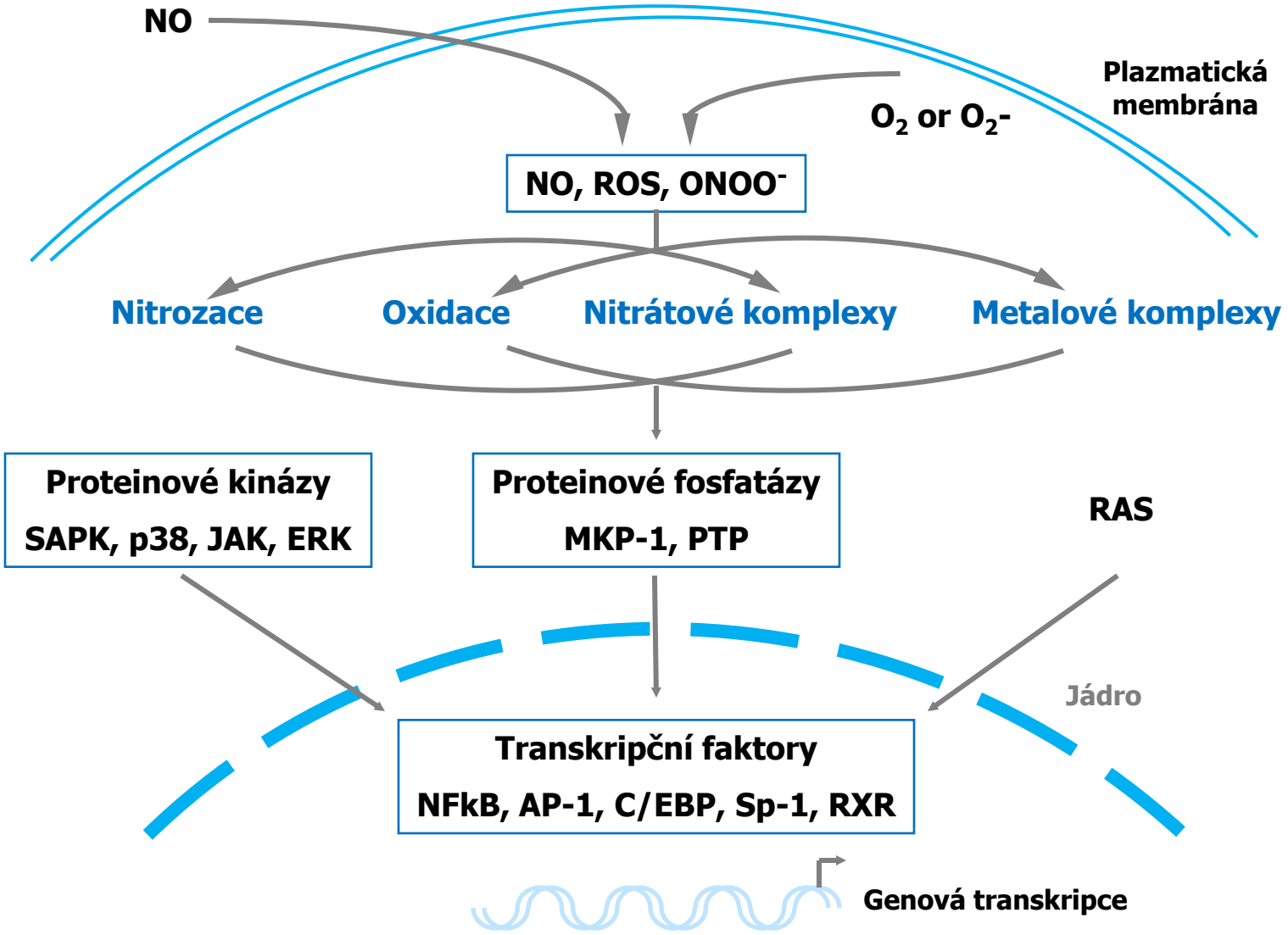
IBD – „inflammatory bowel disease" – zánětlivá onemocnění střeva
NSAIDs – nesteroidní protizánětlivé látky
Vitamíny
Stopové prvky

Hofseth LJ and Ying L, BBA 2006, 1765:74


Oxidativní stres
Aktivace karcinogenů
Trvalý oxidativní stres
Poškození DNA: změny struktury a mutace genů
Inhibice mezibuněčné komunikace
Abnormální genová exprese
Abnormální enzymatická aktivita
Rezistence k chemoterapii
Buněčná proliferace
Apoptóza
Dědičné mutace
Expanze klonů
Metastáze a invazivita
Iniciační stádium
Stádium promoce
Stádium progrese

Schematický přehled úlohy reaktivních kyslíkových radikálů v karcinogenezi. **SOD**, superoxid dismutáza; **.OH**, hydroxylový radikál; **ADF**, adult T-cell leukemia-derived factor; **GTS**, glutathione S-transferase; **GHS**, glutathione.





Hypotetické schéma ilusturující modulaci signálů oxidem dusíku (*NO*) vedoucí ke změně aktivity transkripčních faktorů a exprese genů. (*AP-1* activator protein 1, *ERK* extracellular signal-regulated kinases, *JAK* Janus protein kinases, *MKP-1* mitogen-activated protein kinase phosphatase-1, *NFkB* nuclear factor kB, *NO* nitric oxide, O_2- superoxide, *ONOO*- peroxynitrite, *p38* p38 mitogen-activated protein kinases, *PTP* protein tyrosine phosphatase, *Ras* small GTP-binding protein, *ROS* reactive oxygen species, *RXR* retionid X receptor, *SAPK* stress-activated protein kinases)

Model interakcí indukovaných oxidativním stresem v karcinogenezi

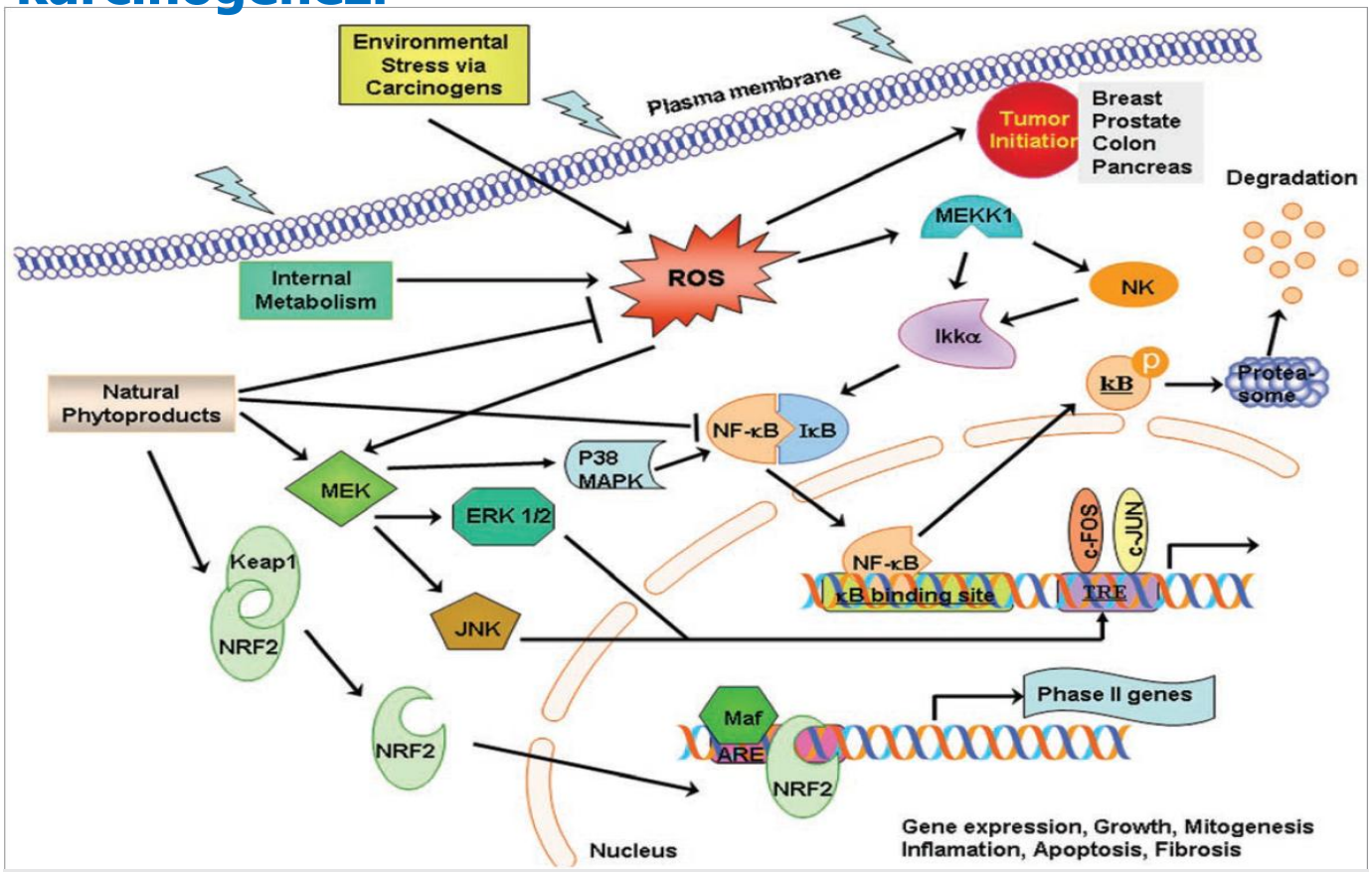


Figure 2. A putative model for oxidative stress induced interactions in carcinogenesis. Reactive oxygen species (ROS) mediated oxidative stress either by environmental carcinogens such as UV and ionizing radiation or by mitochondrial metabolism exerts several effects on DNA damage and generation of oxidative lesions, gene expression, growth regulation mitogenesis, inflammation, apoptosis and fibrosis leading to genomic instability and cancer progression. Chemical signals generated by dietary chemopreventive agents, anticancer agents, antioxidants and antioxidation enzymes, cause Nrf2 nuclear translocation that sets in motion a dynamic machinery of coactivators and corepressors that might form a multimolecular complex with Nrf2 for modulating transcriptional response through the antioxidant response element, ARE producing several phase II detoxification enzymes as stimulation of cellular defense system during stress. Oxidative stress might also cause release of NFκB from IκB and stimulate NFκB nuclear translocation to modulate transcriptional response through the NFκB response element. Several members of the MAPK family may act in concert with Nrf2 and NFκB with multiple interactions between the members of the putative complex to elicit the chemopreventive and pharmacotoxicological events against carcinogenesis.

Environmentální karcinogeny, záření nebo mitochondriální metabolismus indukují oxidativní stres – poškození DNA, změny genové exprese, mitogenezi, zánět, apoptózu a fibrózu vedoucí ke genomové nestabilitě a progresi nádorů.

Acharya A et al. Oxidative medicine and Cellular Longevity 3:23, 2010

Ovlivnění přenosu signálů a účinky ROS na buněčný cyklus a buněčnou smrt. Účinky jsou závislé na dávce a délce expozice

Nekróza

Apoptóza

Zástava bun. cyklu

Diferenciace

Proliferace

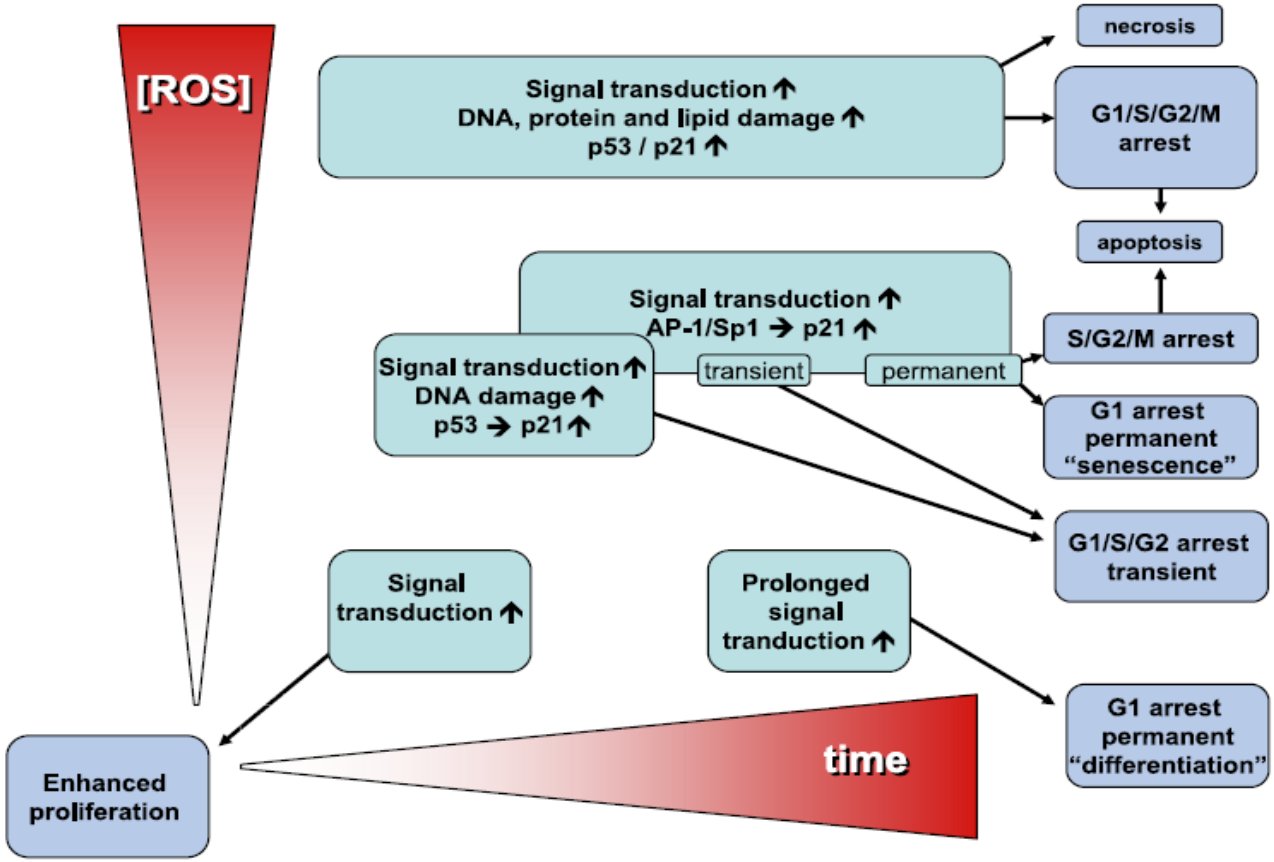
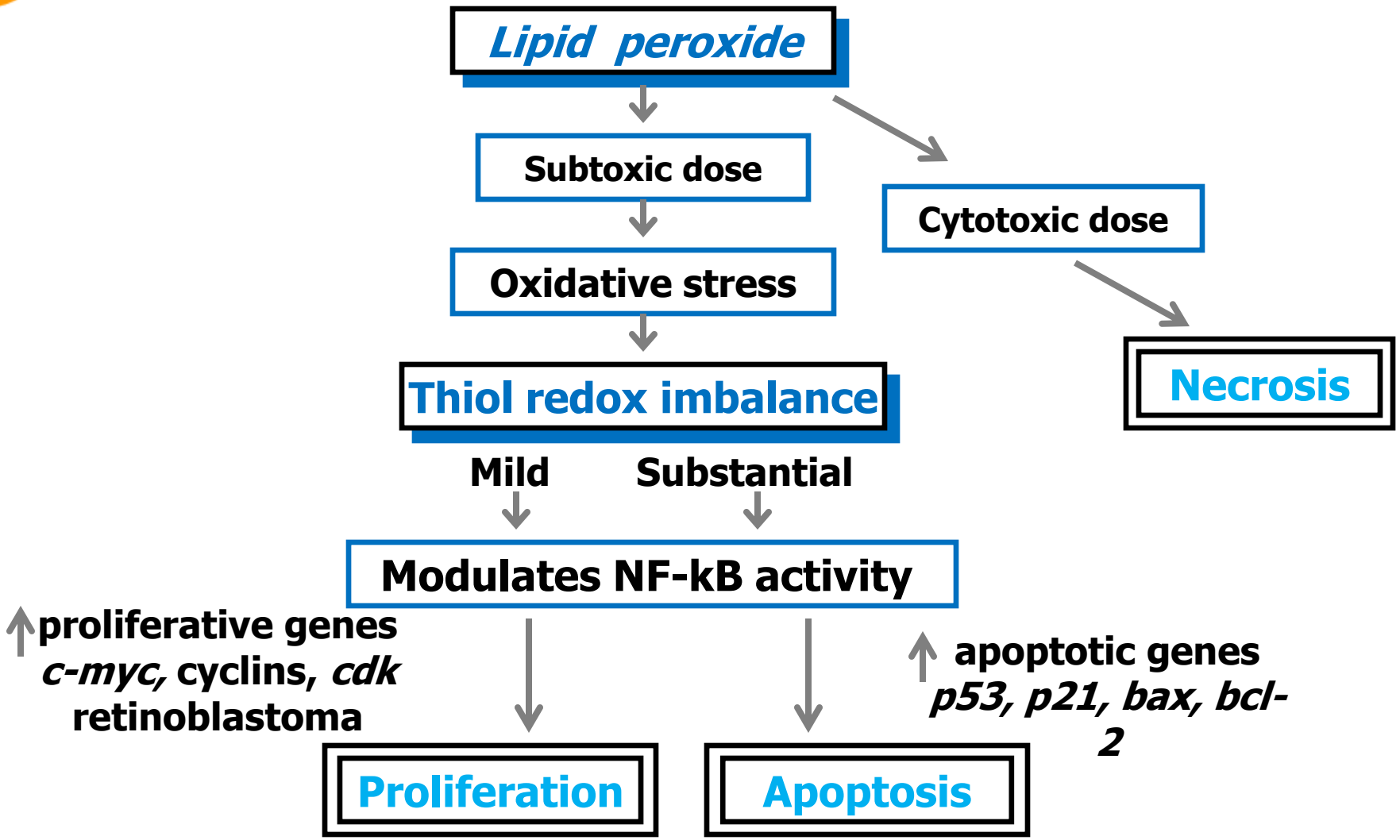


Fig. 3. Scheme, representing the multitude of effects that ROS can have on signal transduction and cell cycle progression. For a given cell and ROS type the effects depend on the amount of ROS and the duration of exposure of the cells to ROS. A short exposure to relatively low doses results in an activation or enhancement of signal transduction pathways leading to (enhanced) cell proliferation. Prolonged exposure to these ROS concentrations will result in prolonged activation of these signal transduction pathways, comparable to the effects of differentiation factors, which will result in a G1 arrest. At higher concentrations and possibly depending on the cellular localization of the ROS, damage to DNA might occur, resulting in an induction of p53 activity and consequently in expression of p21. During the subsequent cell cycle arrest DNA repair will occur after which cell proliferation will resume. Alternatively p21 may become expressed due to the AP-1 or Sp1 sites, which are redox sensitive, resulting in a transient or permanent G1 arrest. If the amounts of ROS are again higher, either due to increase concentrations or prolonged exposure, all changes described above will take place, together with structural damage to proteins and lipids. Under these conditions, cells will arrest in all phases of the cell cycle, especially in the G1 and G2 phases and the cells will undergo apoptosis. Upon sever damage the cells may directly undergo necrosis.

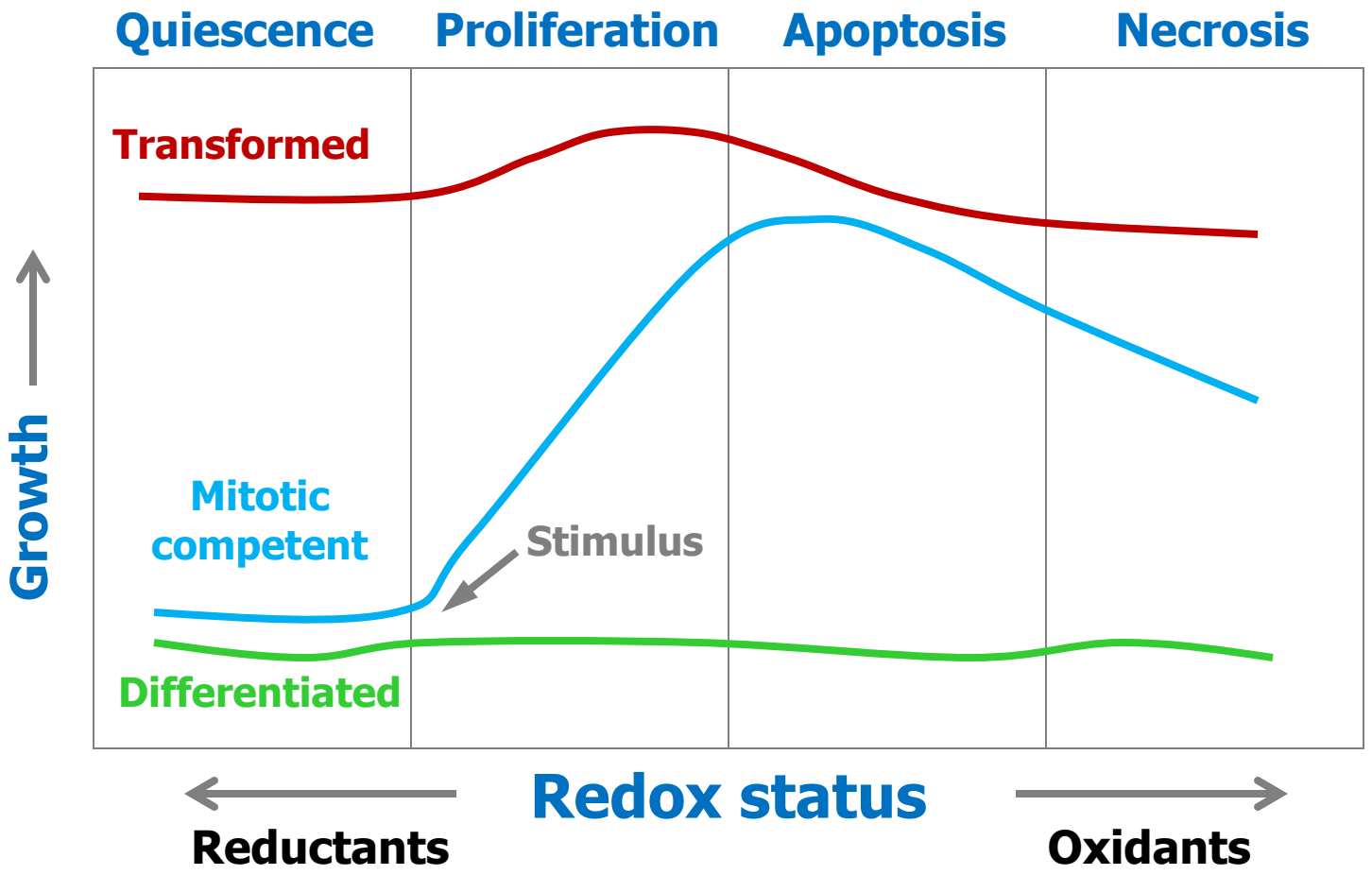
Boonstra and Post, Gene 2004

Oxidativní stres a redoxní nerovnováha ve střevě



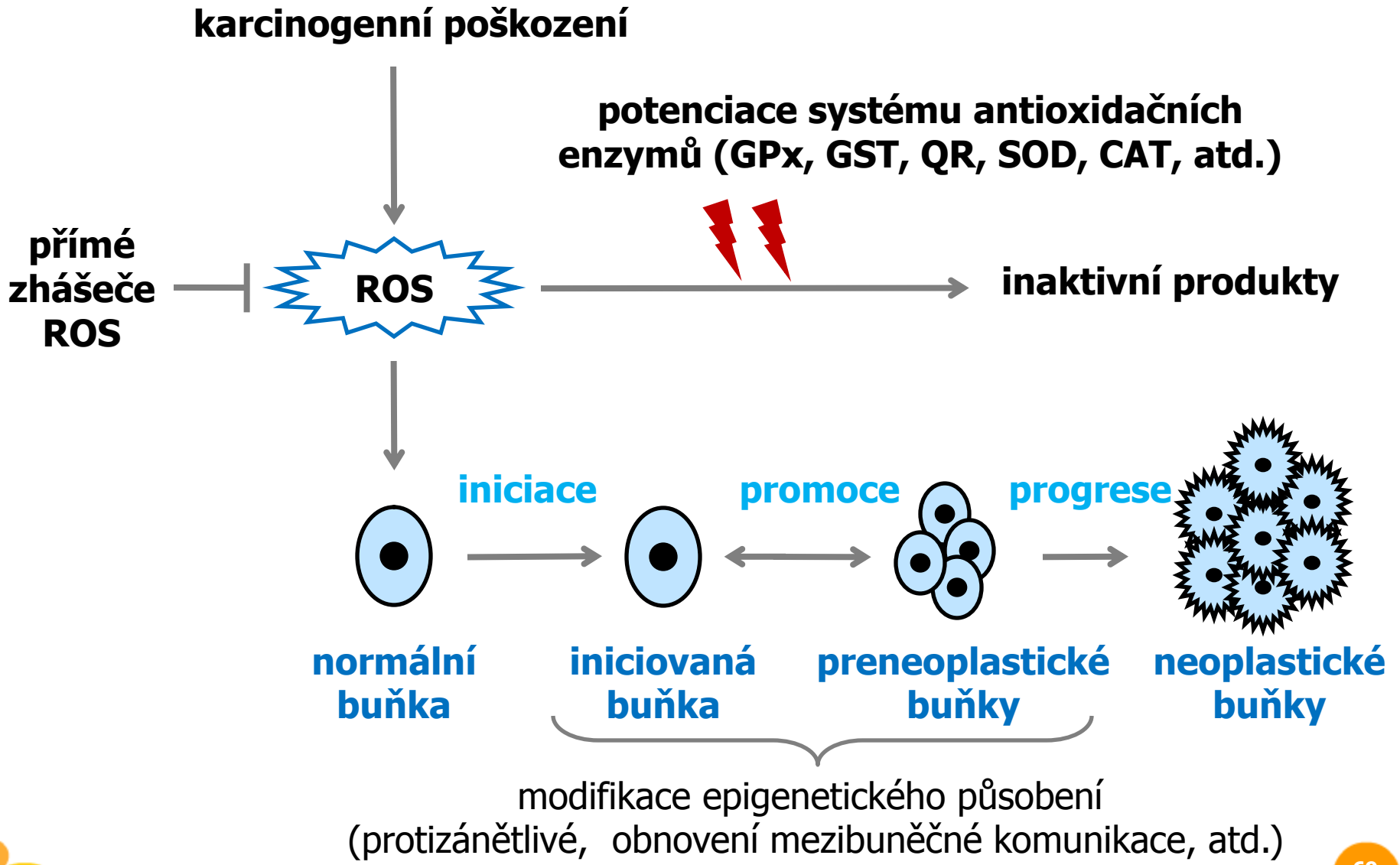
Hypotéza buněčné proliferace a apoptózy indukované lipidovou peroxidací. NF-kB, jaderný transkripční faktor kB.

Oxidativní stres a redoxní nerovnováha ve střevě

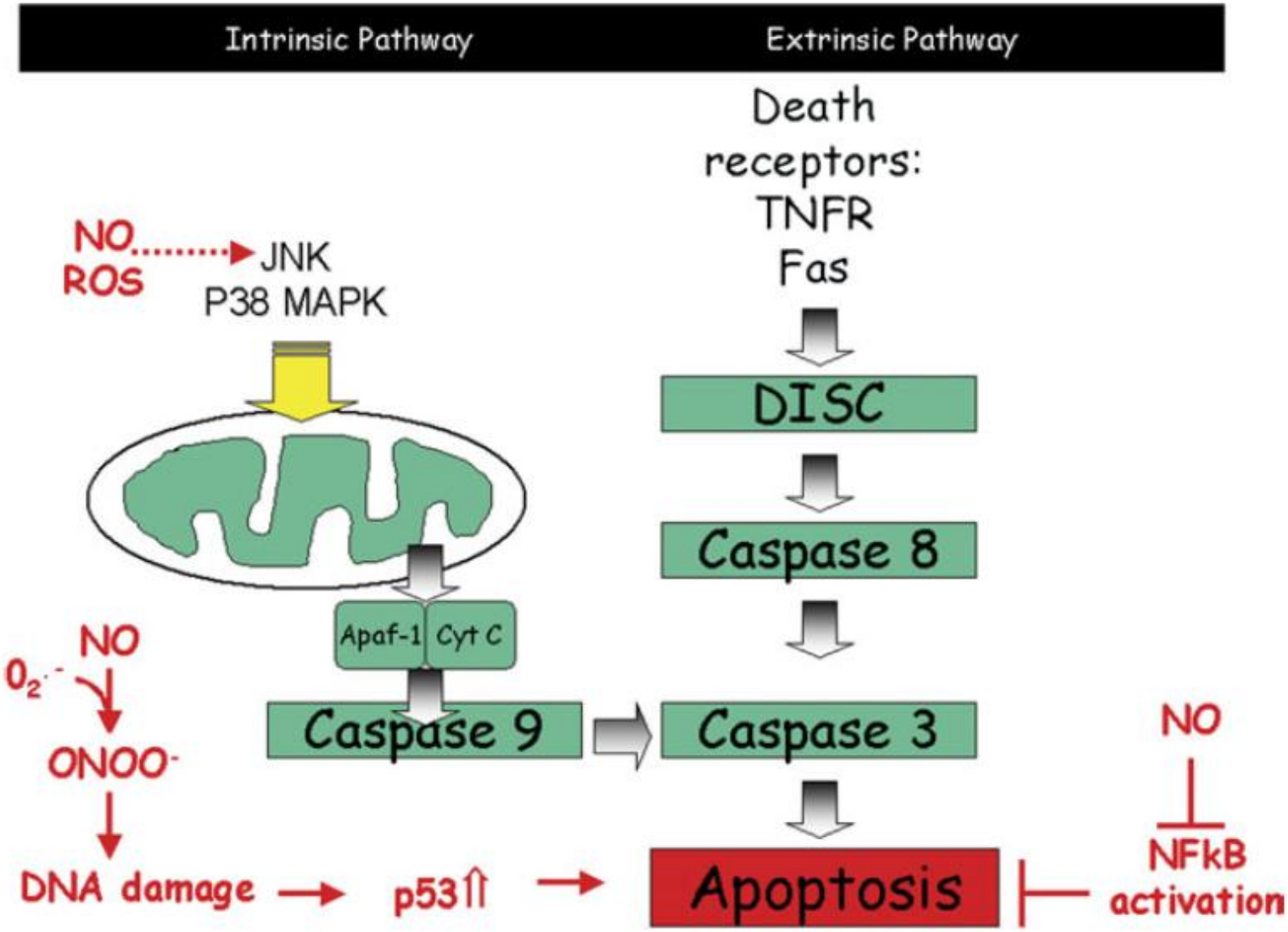


Buněčná odpověď na oxidativní stres a oxidačně-redukční (redox) stav. Křivky představují terminálně diferencované, mitoticky kompetentní a transformované buněčné typy.

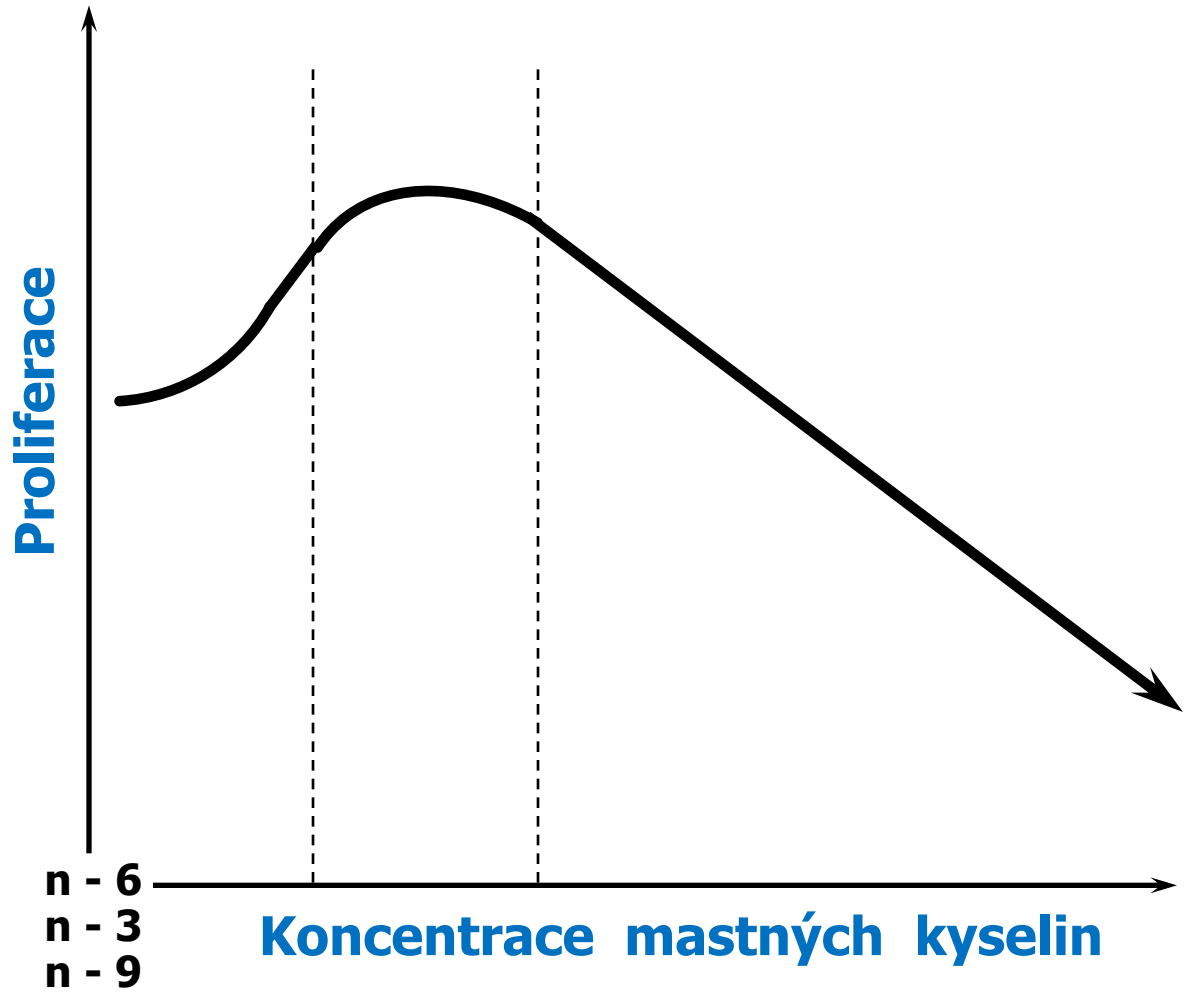
Pravděpodobný mechanismus chemopreventivního účinku vitamínu C v karcinogenezi



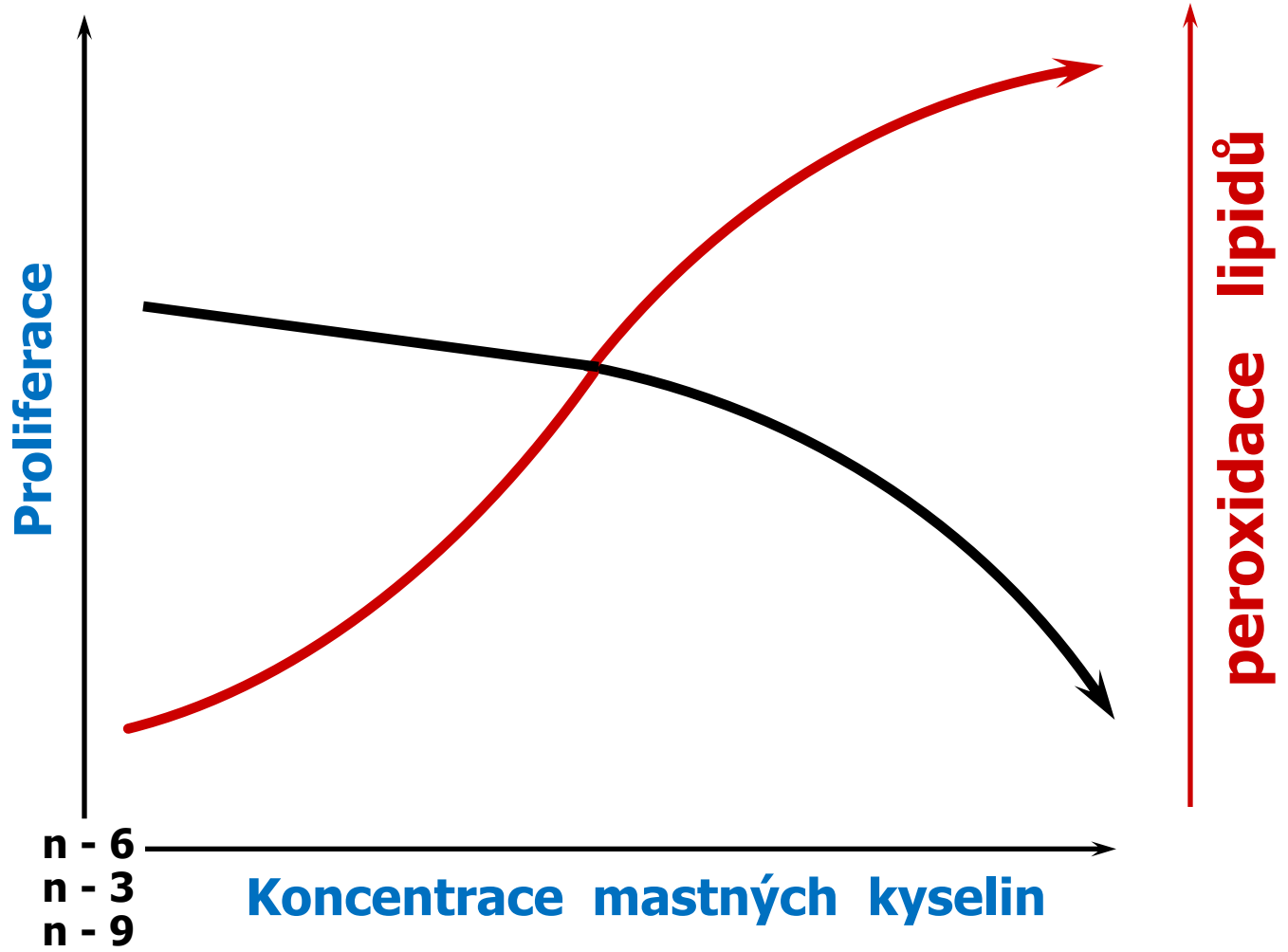
Význam NO a ROS pro vnější a vnitřní dráhu regulující apoptózu



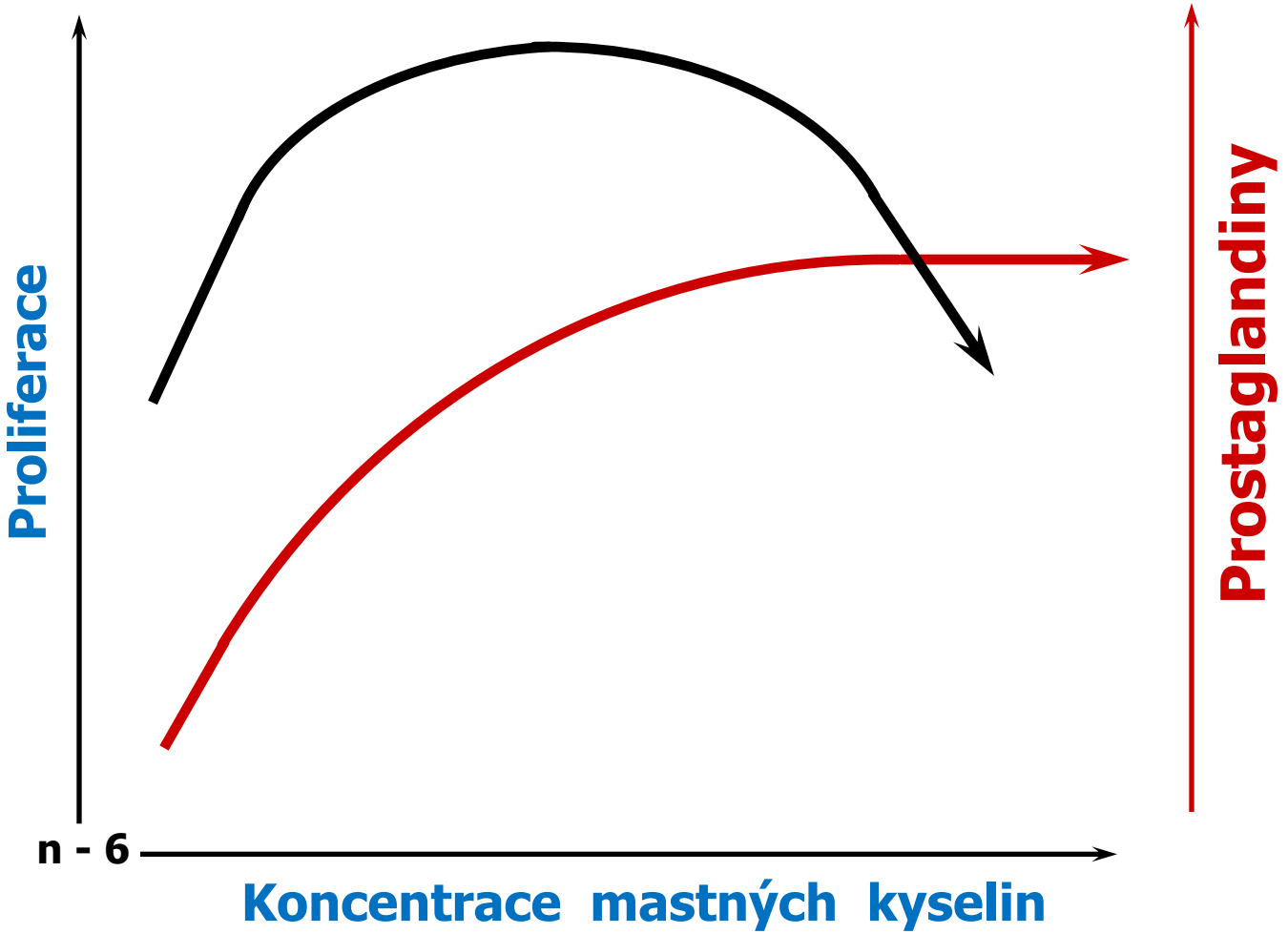
Mastné kyseliny a oxidativní metabolismus



D.G.Cornwell and N.Morisaki, Free Radicals in Biology. Vol.6, 1984



D.G.Cornwell and N.Morisaki, Free Radicals in Biology. Vol.6, 1984



D.G.Cornwell and N.Morisaki, Free Radicals in Biology. Vol.6, 1984

Úloha fosfolipáz v oxidativním stresu, uvolňován kyseliny arachidonové a tvorba prostaglandinů

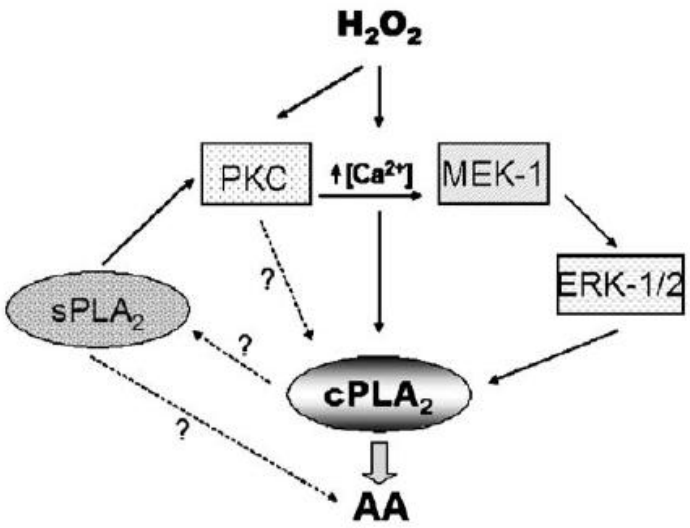


Fig. 1. Role of Ca^{2+}-dependent PLA_2s in oxidative stress. H_2O_2 exposure of the cells results in the activation of intracellular kinase cascades and Ca^{2+} mobilization. Both of these signals converge at the $cPLA_2\alpha$, which is the dominant enzyme in AA mobilization. When present, $sPLA_2$ may amplify AA release by potentiating $cPLA_2$ activation, perhaps via PKC. Alternatively, $cPLA_2\alpha$ may modulate the action of $sPLA_2$, and the latter may also directly effect AA release by directly hydrolyzing membrane phospholipids. PKC, protein kinase C; ERK1/2, extracellular signal-regulated kinases 1/2; MEK, ERK kinase.

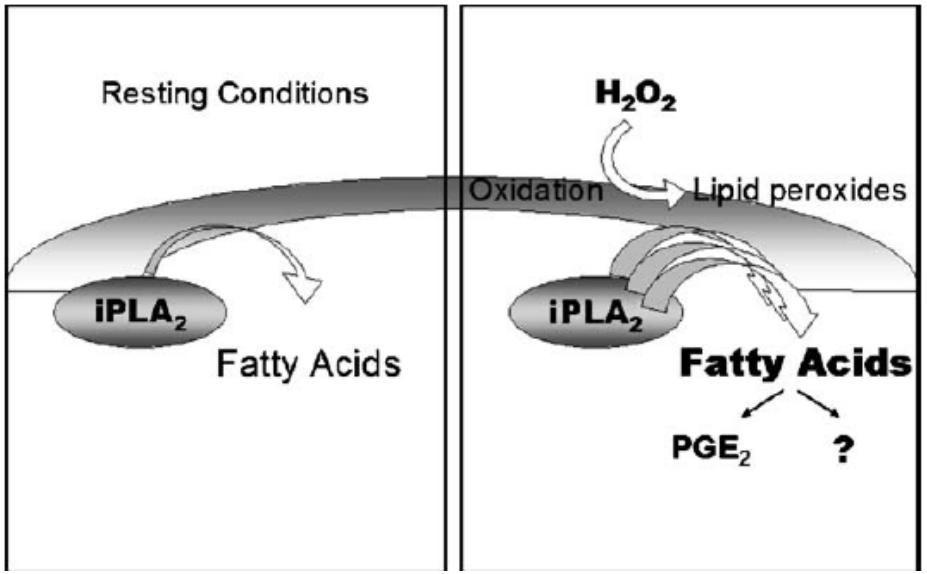
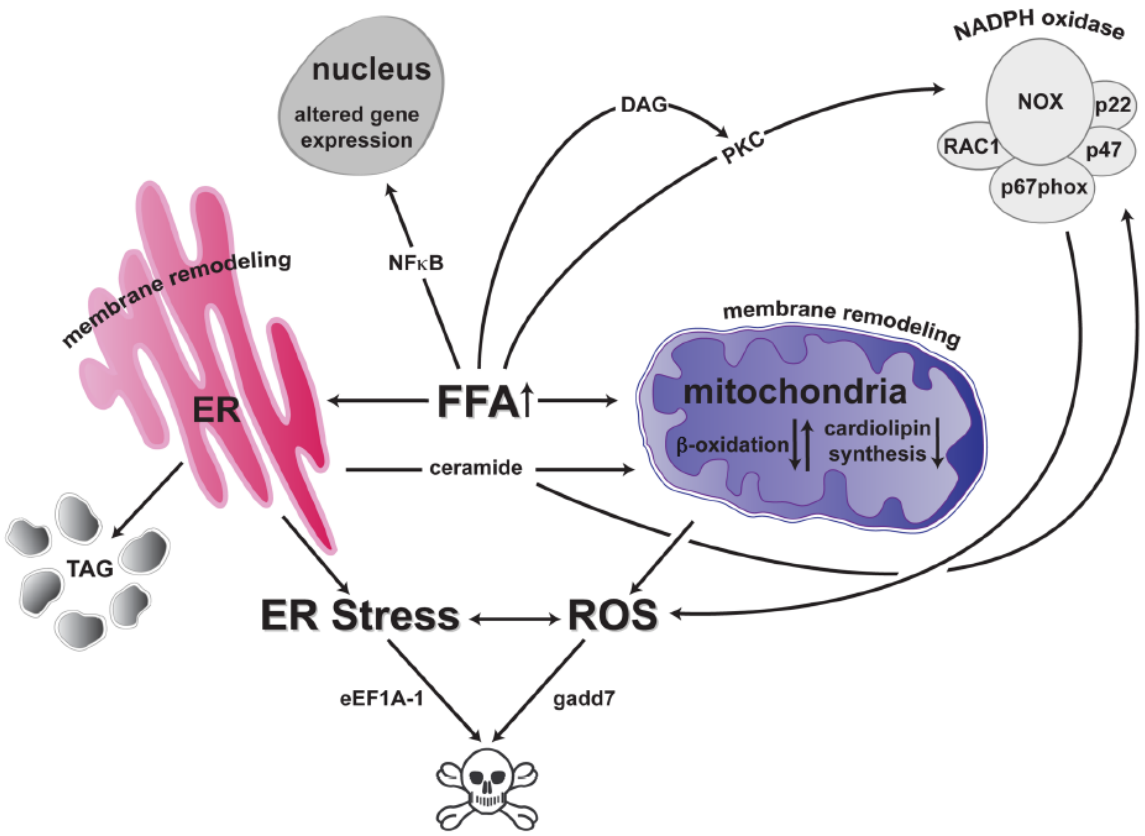


Fig. 2. Role of $iPLA_2$ in oxidative stress. The continuing action of $iPLA_2$ membrane phospholipids under resting conditions helps maintaining the steady-state levels of lysoPC and results the liberation of low levels of free fatty acids such as AA (left panel). When the cells are exposed to H_2O_2, the hydrolytic activity of the $iPLA_2$ increases as result of lipoperoxidation, this leading to increased levels of free AA. Free AA under these conditions may be utilized for prostaglandin synthesis or be used for other cellular functions (right panel).

Lipotoxicita



V netukových buňkách způsobuje nadbytek SFA oxidativní a ER stres způsobený lipidovými metabolity a signálními drahami.

Dysfunkce mitochondrií a ER stres jsou klíčové děje, jimiž je při nadbytku lipidů indukována buněčná smrt.

Nasměrování nadbytečných mastných kyselin do lipidových dropletů má ochranné účinky.

Figure 1. The lipotoxic-response
In non-adipose cells, excess saturated FFAs induce oxidative and ER stress through lipid metabolites and signaling pathways. Dysfunction of mitochondria and the ER are key steps through which excess lipid induces cell death, whereas channeling of excess FFAs to lipid droplets is cytoprotective.

Brookheart RT et al Cell Metab 2009, 10:9

Působení nasycených (SA) a nenasycených (MUFA) mastných kyselin na rozdělení lipidů a lipotoxicitu

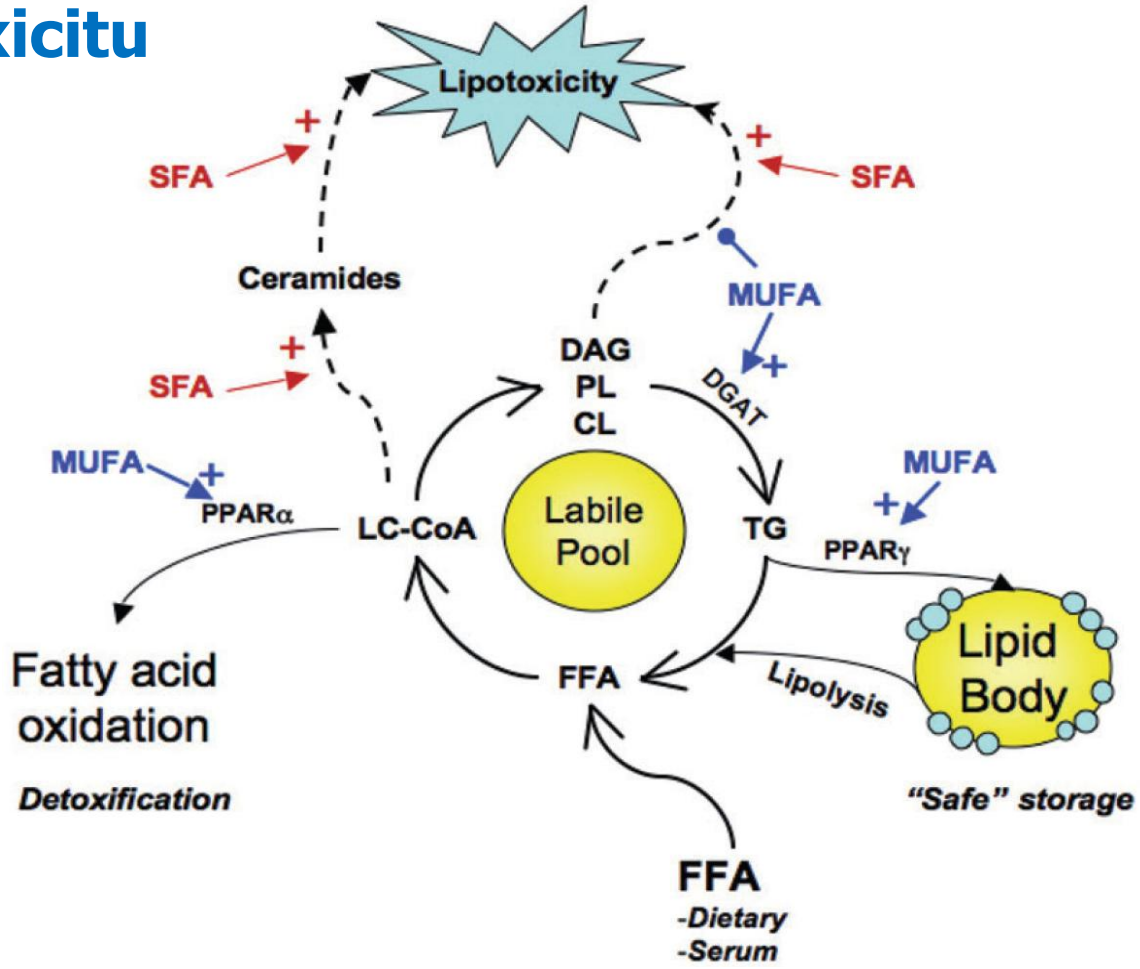


Figure 1 Model depicting effects of saturated fatty acids (SFA) compared to monounsaturated fatty acids (MUFA) on lipid partitioning and lipotoxicity. SFA are less well incorporated into triglycerides (TG) than are MUFA, as the enzyme diacylglycerol acyltransferase (DGAT) preferentially incorporates monounsaturated acyl-chains. SFA are also required for ceramide synthesis. SFA lead to greater accumulation of diacylgycerides (DAG) and a pattern of phospholipids (PL) with reduced cardiolipin (CL) production. This SFA pattern of lipid partitioning is associated with greater lipotoxicity. MUFA are well incorporated into TG and into lipid droplets that form a safe means of lipid storage. In this way fatty acids are removed from the functionally active labile pool of lipids. MUFA rather than SFA also activate the nuclear transcription factors PPARα and PPARγ which respectively promote lipid detoxification via fatty acid oxidation and safe fatty acid storage. Importantly, MUFA promote the safer partitioning of SFA into TG and fatty acid oxidation pathways.

Nolan ChJ and Larter CZ J Gastroenterol Hepatol 2009, 24:830

Receptory pro peroxisomové proliferátory (PPARs)

Mastné kyseliny a jejich metabolity fungují jako aktivátory PPARs

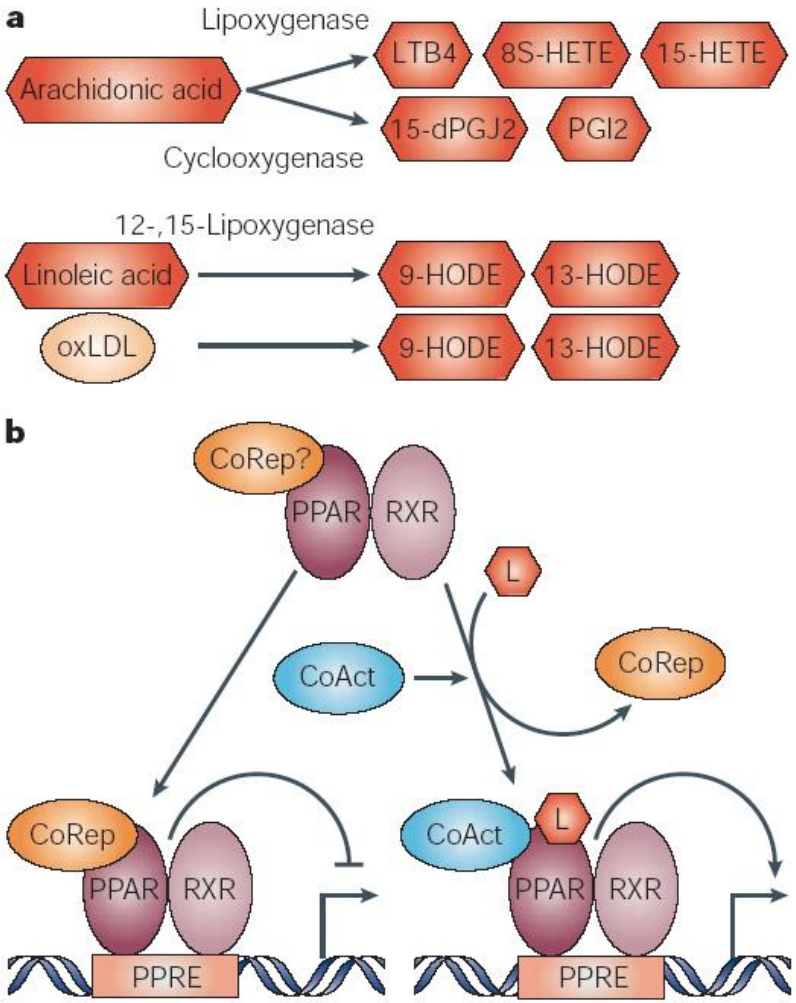
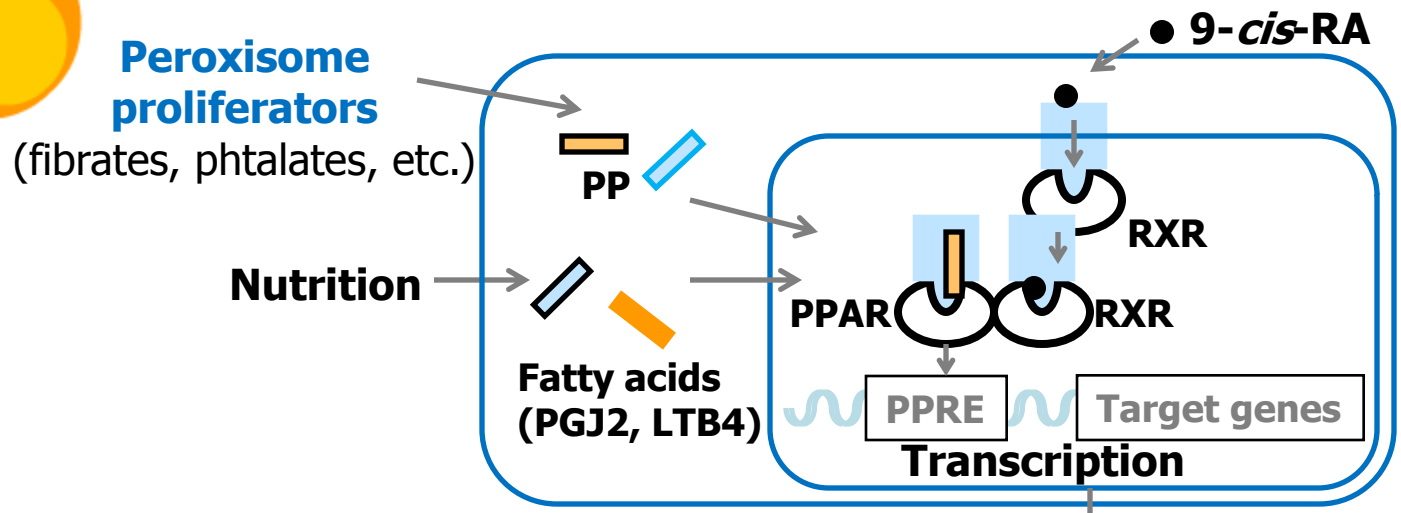


Figure 1 | **Schematic representation of the PPAR signalling pathways. a** | Endogenous agonists of peroxisome-proliferator-activated receptors (PPARs). PPARs are ligand-inducible receptors, which can be activated by fatty acids — such as arachidonic or linoleic acids — and their derivatives. The fatty-acid metabolites that activate PPARs are mainly derived from arachidonic or linoleic acids through the cyclooxygenase or the lipoxygenase pathways. The best characterized at the moment are leukotriene B4 (LTB4) and 8S-HETE (hydroxyeicosatetraenoic acid), which preferentially activate PPARα; 15-deoxy-prostaglandin J2 (15-dPGJ2) and 15-HETE, which are PPARγ-selective ligands; and the prostaglandin I2 (PGI2, also called prostacyclin), which is probably a PPARβ/δ natural ligand. PPARγis also activated by 9-HODE (hydroxyoctadecadienoic acid) and 13-HODE, either derived from linoleic acid or as components of oxidized low-density lipoprotein (oxLDL). **b** | PPARs function as heterodimers with their obligate partner, retinoid receptor (RXR). The dimer probably interacts with co-regulators, either co-activators (CoAct) or co-repressors (CoRep). In the unliganded form, PPARβ/δ–RXR heterodimer, in contrast to PPARα–RXR and PPARγ–RXR heterodimers, recruits co-repressors and represses the activity of PPARα and PPARγ target genes by binding to the peroxisome proliferator response element (PPRE) that is present in their promoters[6,7]. In their liganded form, the PPAR–RXR heterodimers interact with co-activators, bind to the PPRE that is present in the promoters of their target genes and activate their transcription.

Michalik L et al., Nature Rev Cancer 2004



Cell specific responses

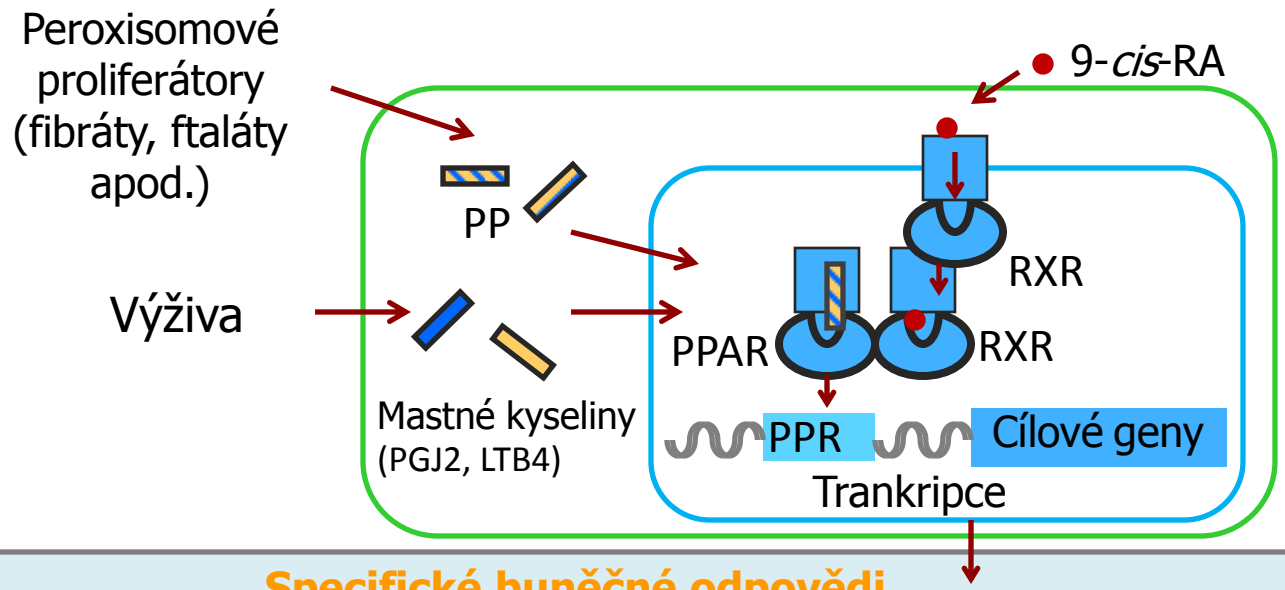
Proliferation	Differentiation and maturation	Apoptosis

Medical relevance

•Clonal expansion of preadipocytes pro-moting adipogenesis (participation on PPARg.) •Hypothetical risk in man of cell growth stimulation by activation of PPARs.	•Monocyte / macro-phage differentiation (implication of PPARg) leading to accelerated atherosclerosis. •Protective effects of PPARa. •Adipocyte differen-tiation responsible of obesity and other related disorders (implication of PPARa.)	•Enhanced PPARg expression could lead to tumoral cell apoptosis and represents a therapeutical approach in malignant disease.

Importance of PPARs in cell proliferation, differentiation and apoptosis.

After activation, PPAR and RXR form heterodimers which bind to DNA regulato-ry sequences of target genes through interaction with PPRE. The control by PPARs of the transcriptional activity af target ge-nes gives rise to bio-logical effects which may have consequen-ces for human health. LTB4, leukotriene B4; PGJ2, prostagladin J2; PP, peroxisome proliferator; PPAR, peroxisome prolifera-tor-activated receptor; PPRE, peroxisome proliferator respon-sive element; 9-cis-RA, 9-cis-retinoic acid; RXR, 9-cis-retinoic acid receptor.



Specifické buněčné odpovědi

Proliferace	Diferenciace a zrání	Apoptóza
* Klonální expanze preadipocytů podporující adipogenesi (účast PPARγ.) * Hypotetické riziko buněčné růstové stimulace aktivací PPARs.	* Diferenciace monocytů / macrofágů (podíl PPARγ)vedoucí k urychlené ateroskleróze * Ochranné účinky PPARα. * Diferenciace adipocytů odpovědná za obezitu a další poruchy (podíl PPARα.)	* Zvýšená exprese PPARγ by mohla vést k apoptóze nádorových buněk a představuje možný terapeutický protinádorový přístup

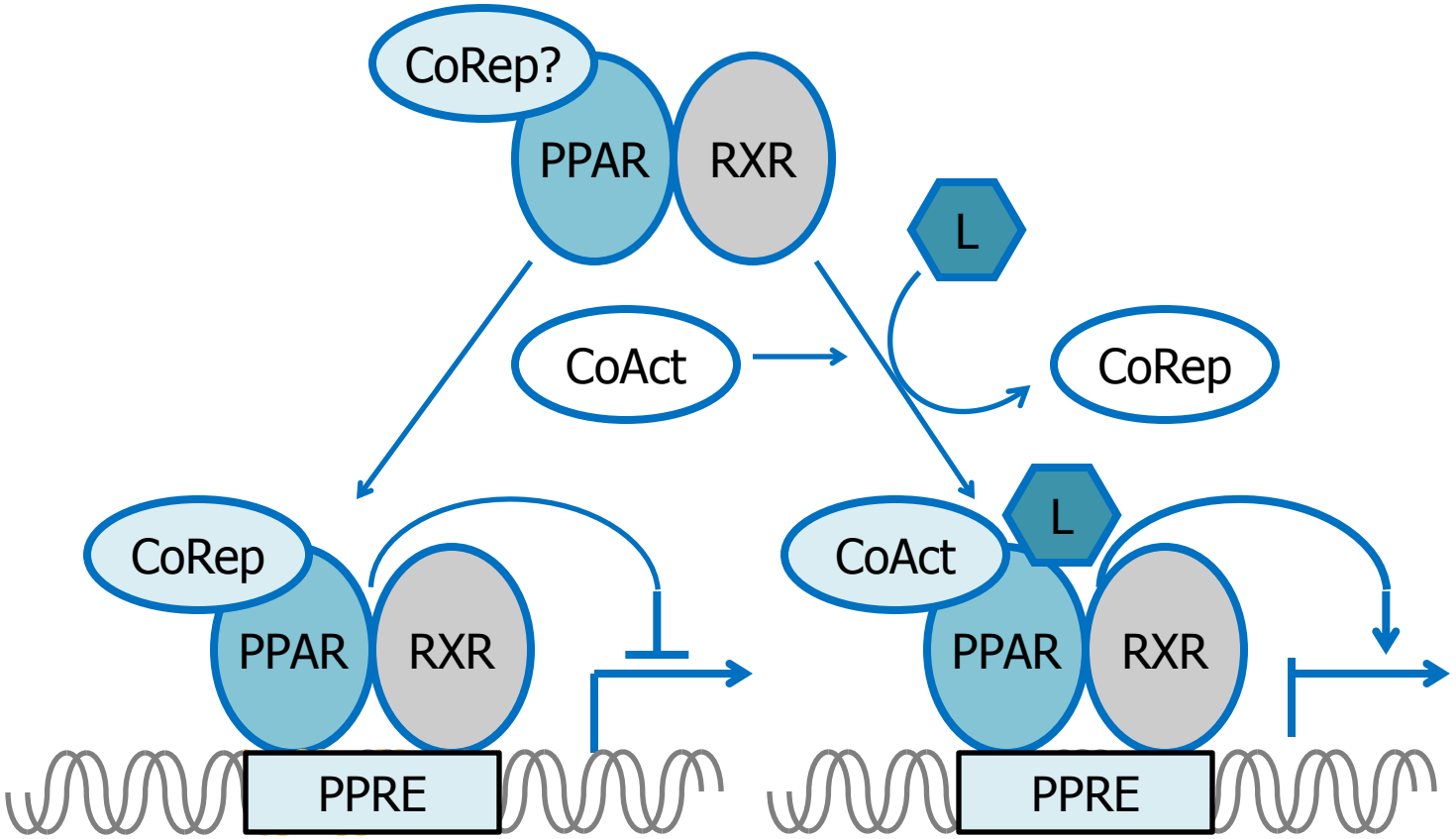
Medical relevance

Důležitost PPARs v buněčné proliferasci, diferenciaci a apoptóze.
Po aktivaci, PPAR a RXR tvoří heterodimery, které se vážou na regulační sekvence cílových genů prostřednictvím PPRE na DNA.

Kontrola transkripční aktivity cílových genů PPAR vede k biologickým účinkům ovlivňujícím lidské zdraví.

LTB4, leukotrien B4; PGJ2, prostagladin J2; PP, peroxisom. proliferátor; PPAR, receptor aktivovaný PP; PPRE, respon-sivní element pro PP; 9-cis-RA, 9-cis-retinová kyselina; RXR, receptor pro 9-cis RA.

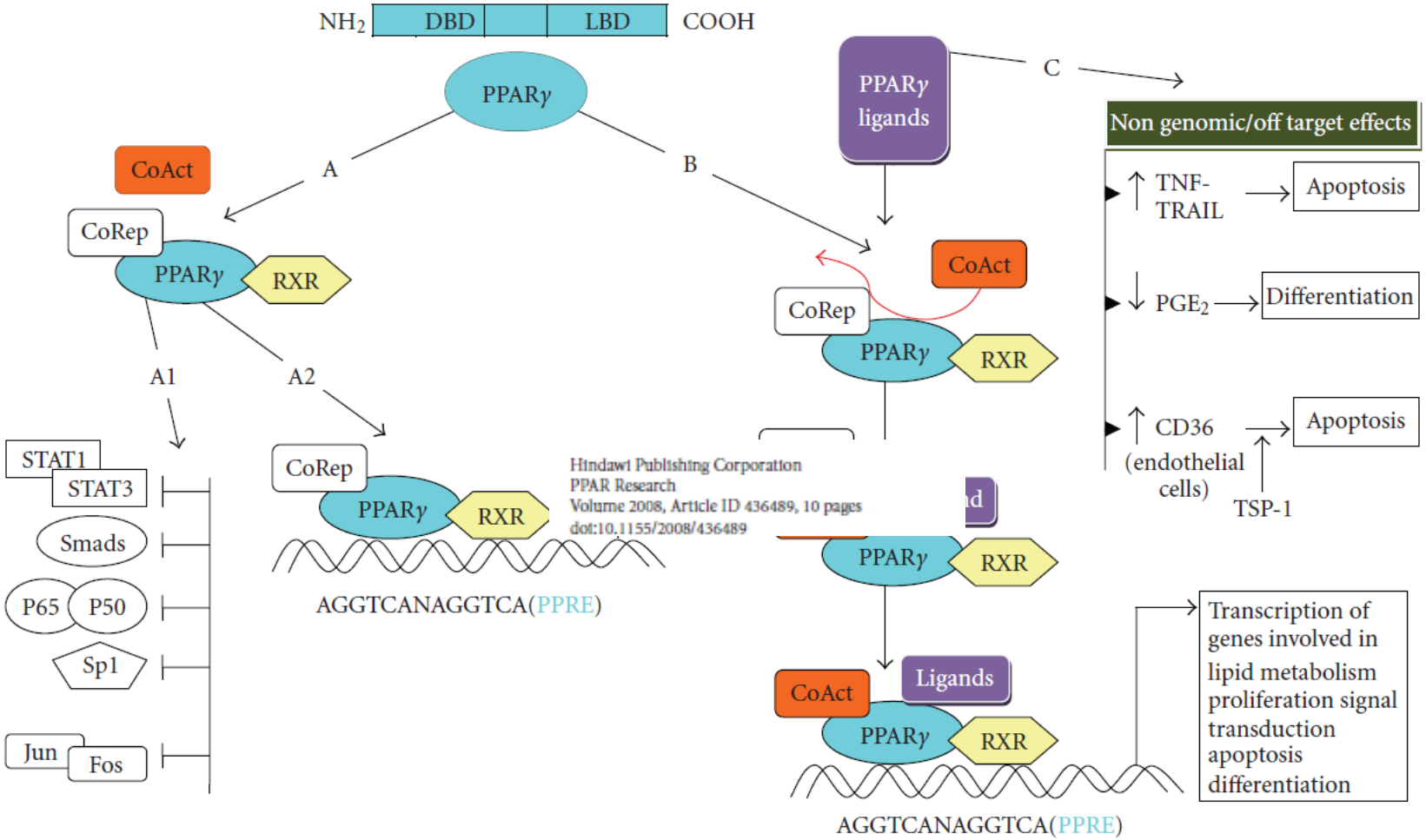
Schéma signálních drah PPAR



PPARs fungují jako heterodimery s jejich obvyklým partnerem – retinoidním receptorem (RXR)

CoRep korepresor, Co Act koaktivátor,RXR receptor pro retinovou kyselinu X, PPRE responsivní element pro PP

Signální dráhy a funkce PPARs a jejich ligandů



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Volume 2008, Article ID 436489, 10 pages
doi:10.1155/2008/436489

FIGURE 1: *Peroxisome proliferator-activated receptor-γ and ligands: pathways and functions.* PPARγ protein exhibits a structural organization consisting of three functional domains: an N-terminal domain, a DNA-binding domain (DBD) and a carboxy-terminal ligand binding domain (LBD). PPARγ forms heterodimers with a second member of the nuclear receptor family, the retinoic X receptor (RXR). Unliganded PPARγ suppresses transcription (pathway A) either by interfering with key transcription factors (pathway A1) or through recruitment of corepressors (CoRep) on a PPRE element (pathway A2). Ligand binding to PPARγ (pathway B) triggers conformational changes that lead to dissociation of corepressors (CoRep) and subsequent association of coactivators (CoAct). The complex is binding to PPREs and triggers transcription (pathway B). PPARs ligands can also exert their action through PPARγ-independent mechanisms also (pathway C). For instance in NSCLC cell lines activation of TNF-TRAIL induce apoptosis, while PGE_2 degradation, trough 15-hydroxyprostagladin dehydrogenase induction, results in enhanced epithelial differentiation. In endothelial cells PPARγ ligands can markedly boost expression of CD36 which functions as the receptor of endogenous antiangiogenic molecule thrombospondin-1, thereby potentiating the apoptotic response. (PFAs: polyunsaturated fatty acids, TZDs: thiazolidinediones, PPRE: peroxisome proliferator response element, TNF: tumor necrosis factor, TRAIL: TNF-related apoptosis-inducing ligand, NSCLC: non-small cell lung carcinoma).

Sainis I et al., PPAR Research 2008

Změny korepresorů/koaktivátorů závislé na ligandu

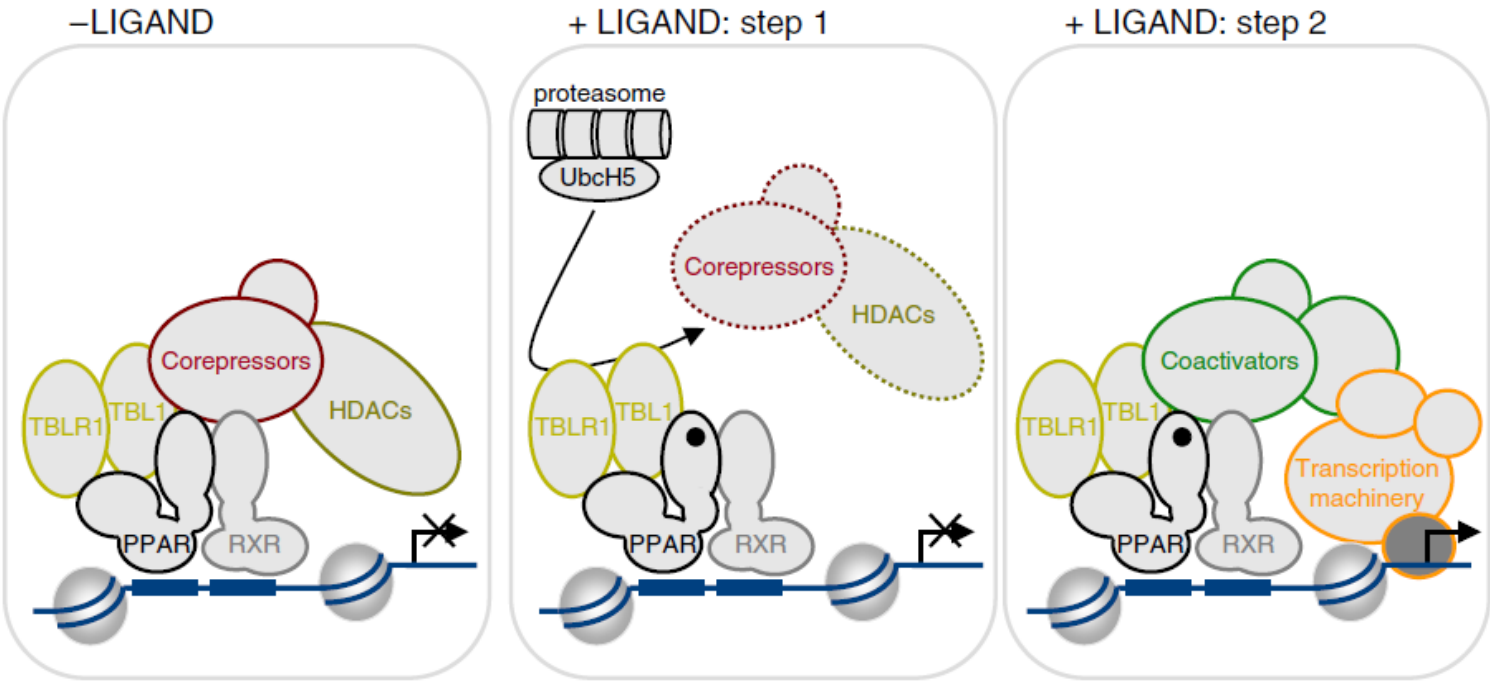
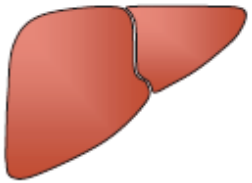
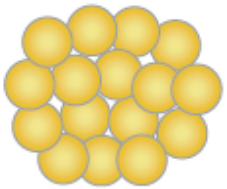


Fig. 2. Ligand-dependent corepressor/coactivator exchange. Certain PPAR target genes are maintained in an inactive state in the absence of ligand by the constitutive association of a PPAR/RXR heterodimer bound to corepressors (−LIGAND). These corepressors associate with histone deacetylases (HDACs) which maintain histone tails in a hypoacetylated state. The nuclear corepressor exchange factors (NCoEx) TBL1 and TBLR1 are also bound to the receptors. Upon ligand binding, NCoEx recruit the E2 ubiquitin conjugating enzyme UbcH5 and the ubiquitylation machinery to induce ubiquitylation and subsequent proteasomal degradation of corepressors (+LIGAND: step 1). Thus, NCoEx facilitate the clearance of corepressors, which allows efficient coactivator recruitment and transcriptional activation (+LIGAND: step 2). This model is derived from [26,124].

Feige JN et al., Progress in Lipid Res 2006, 45:120

Hlavní metabolické funkce regulované PPARs





		LIVER	MUSCLE	ADIPOSE TISSUE
Lipid utilization	PPARα	- Fatty acid oxidation - Response to fasting	- Fatty acid oxidation - Energy uncoupling	
	PPARβ		- Fatty acid oxidation - Energy uncoupling	- Fatty acid oxidation - Energy uncoupling
Lipid storage & Insulin sensitivity	PPARγ	- Lipogenesis - Insulin sensitivity	- Insulin sensitivity	- Adipocyte differentiation - Adipocyte survival - Lipogenesis - Adipokine secretion - Insulin sensitivity

Využití a metabolizmus lipidů, ukládání lipidů a citlivost k inzulinu

Feige JN et al., Progress in Lipid Res 2006, 45:120

Funkce PPARs ve vztahu ke karcinogenezi

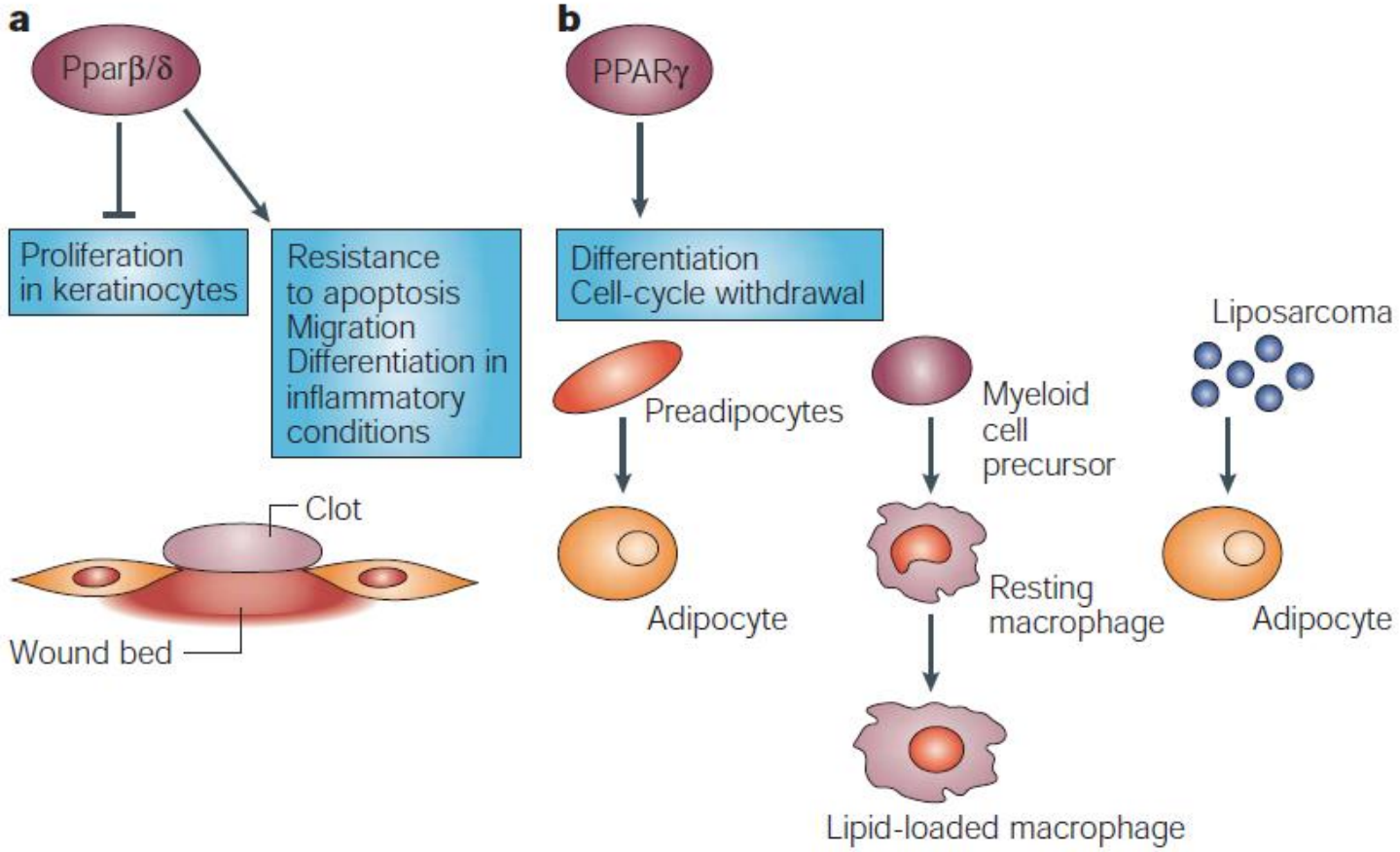
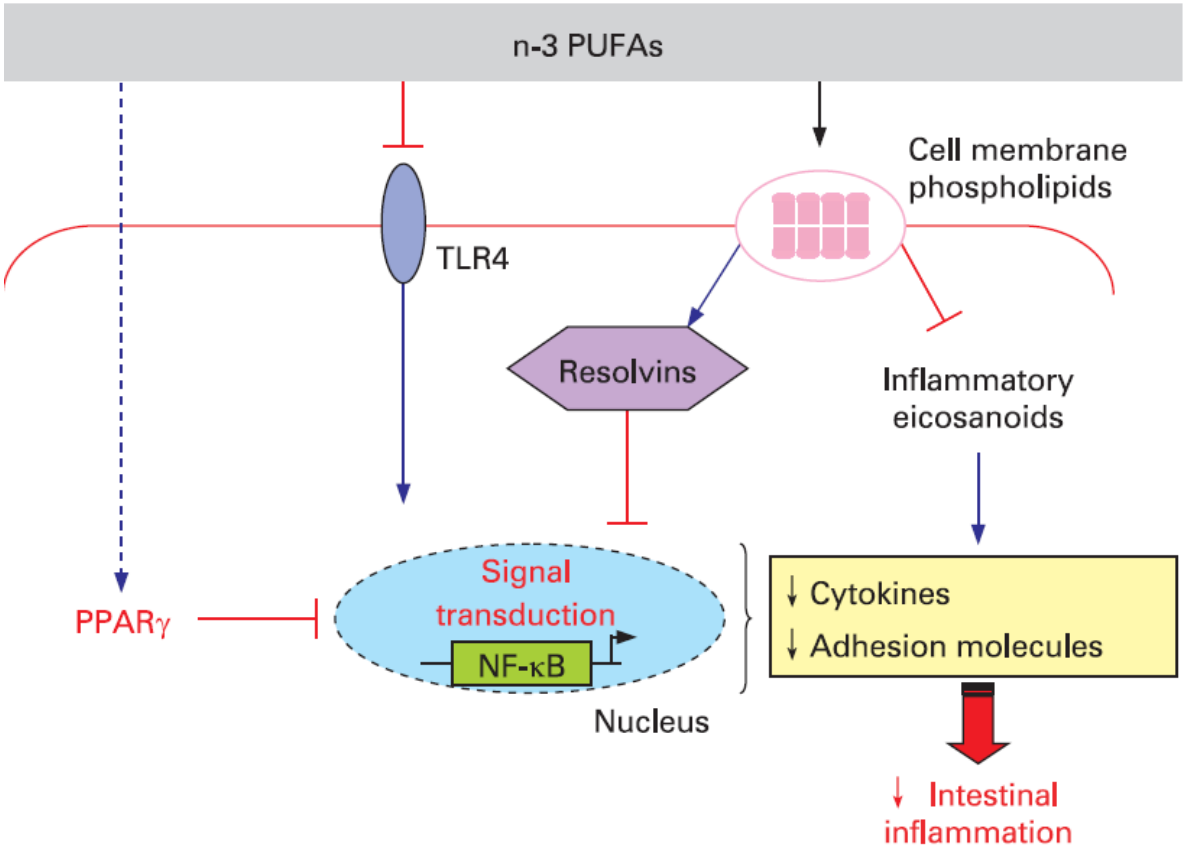


Figure 3 | **PPARβ/δ and PPARγ functions that relate to their carcinogenic properties.** **a** | As demonstrated in a mouse-skin wound-healing model, Pparβ/δ inhibits keratinocyte proliferation and participates in inflammation-induced keratinocyte differentiation, which are anti-carcinogenic actions. However, it also increases both migration and keratinocyte resistance to Tnf-α-induced apoptosis. **b** | PPARγ is implicated in the differentiation of pre-adipocytes to adipocytes and of monocytes to macrophages. In the presence of PPARγ and retinoid receptor (RXR) ligands, myeloid-cell precursors become resting macrophages, which can be turned to lipid-loaded macrophages, when PPARγ and RXR ligands are maintained. PPARγ can also withdraw liposarcoma-derived cells from cell division to trigger their differentiation to adipocytes.

Michalik L et al., Nature Rev Cancer 2004

Mechanizmy působení n-3 PUFAs v zánětu střeva



N-3 PUFAs aktivují PPARγ, který inhibuje signální dráhu NFκB a mohou inhibovat toll-like receptor 4 (TLR4).
Mohou také modulovat složení membránových fosfolipidů vedoucí ke snížení produkce prozánětlivých eikosanoidů odvozených od AA a zvýšení produkce protizánětlivých resolvinů.
Tyto regulační dráhy snižují produkci prozánětlivých cytokinů a expresi adhezívních molekul
To vede ke snížení zánětu střeva.

Marion-Latellier R et al., Gut 2009, 58:586

Přírodní zdroje modulátorů PPARγ a účinky u zánětlivých onemocnění střeva

Table 3 Peroxisome proliferator-activated receptor gamma (PPARγ) modulators which have shown some efficacy in inflammatory diseases of the human intestine

Nutrient	Dose and duration	Patients	Main results
Butyrate[92]	4 g/day for 8 weeks	13 patients with mildly to moderately active Crohn's disease	Decrease of CDAI after 4 and 8 weeks of treatment Decrease of mucosal levels of NF-κB and IL1β
Curcumin[64]	1.1 g/day for 1 month	5 patients with ulcerative colitis, and 5 with Crohn's disease	Reduction of concomitant medications for 4/5 patients with ulcerative colitis
	1.65 g/day for 1 month		Reduction of CDAI score for 4/5 patients with Crohn's disease
Curcumin[93]	2 g/day for 6 month	89 patients with quiescent ulcerative colitis: 45 curcumin/44 placebo	Reduction of relapse rate Improvement of CAI and EI
Saccharomyces boulardii[94]	1 g/day for 6 months	32 patients with Crohn's disease in clinical remission	Reduction of clinical relapse rate
VSL#3[95]	2 sachets/day for 4 weeks	Ileal pouch–anal anastomosis for ulcerative colitis	Reduction of PDAI score Reduction of IL1β mRNA expression
VSL#3[96]	2 sachets/day for 4 weeks	23 patients with active mild pouchitis (ulcerative colitis)	16/23 patients in remission Decrease of PDAI score Improvement in the quality of life

Table 2 Dietary sources of natural peroxisome proliferator-activated receptor gamma (PPARγ) modulators

Name	Dietary source
α-Linolenic acid	Leafy green vegetables, flax
Capsaicin	Cayenne pepper
Conjugated linoleic acid	Beef, bovine milk
Curcumin	Turmeric powder
Docosahexaenoic acid	Fish
Eicosapentaenoic acid	Fish
Epigallocatechin gallate	Green tea
γ-Linolenic acid	Vegetable oils and blackcurrant
Ginsenosides	Ginseng
Hesperidin	Citrus fruits
Kochujang	Korean fermented red pepper paste
ψ-Baptigenin	Plants (red clover and hen's eye)
Resveratrol	Grapes, wine, peanuts

Marion-Latellier R et al., Gut 2009, 58:586

Mutace PPARγ v lidských nádorech a účinky agonistů PPARγ u různých buněčných typů

Table 1 | **Mutations found in PPARγ in human tumours**

Tissue	Frequency	Mutation	References
Sporadic colon tumors	4/55	Loss of function	112
Follicular thyroid carcinomas	5/8	Chromosomal translocation; PAX8–PPARγ fusion protein; dominant-negative inhibitor of PPARγ	113
Follicular thyroid carcinomas	5/9	Chromosomal translocation; PAX8–PPARγ fusion protein	114
Follicular thyroid adenomas	2/16	Dominant-negative inhibitor of PPARγ	
Follicular thyroid carcinomas Follicular thyroid adenomas	13/33 1/23	Chromosomal translocation; PAX8–PPARγ fusion protein	115
Follicular thyroid carcinomas Follicular thyroid adenomas	6/17 6/11	Chromosomal translocation; PAX8–PPARγ fusion protein	116
Prostate tumours	8/38	Hemizygous deletion of PPARγ	59
71 cancer cell lines; 326 clinical cancers (colon, prostate, breast,lung cancers, osteosarcomas, leukaemias)	0	No mutation found	117

Mutations that decreased the activity of the receptor were described in the *PPARγ* gene in some human tumours of various origins. Overall, these mutations are rather rare, indicating that decreased activity of PPARγ might contribute to carcinogenesis, but is probably not causal to the pathology. For the sake of clarity (different approaches), each publication cited in the table is listed separately.

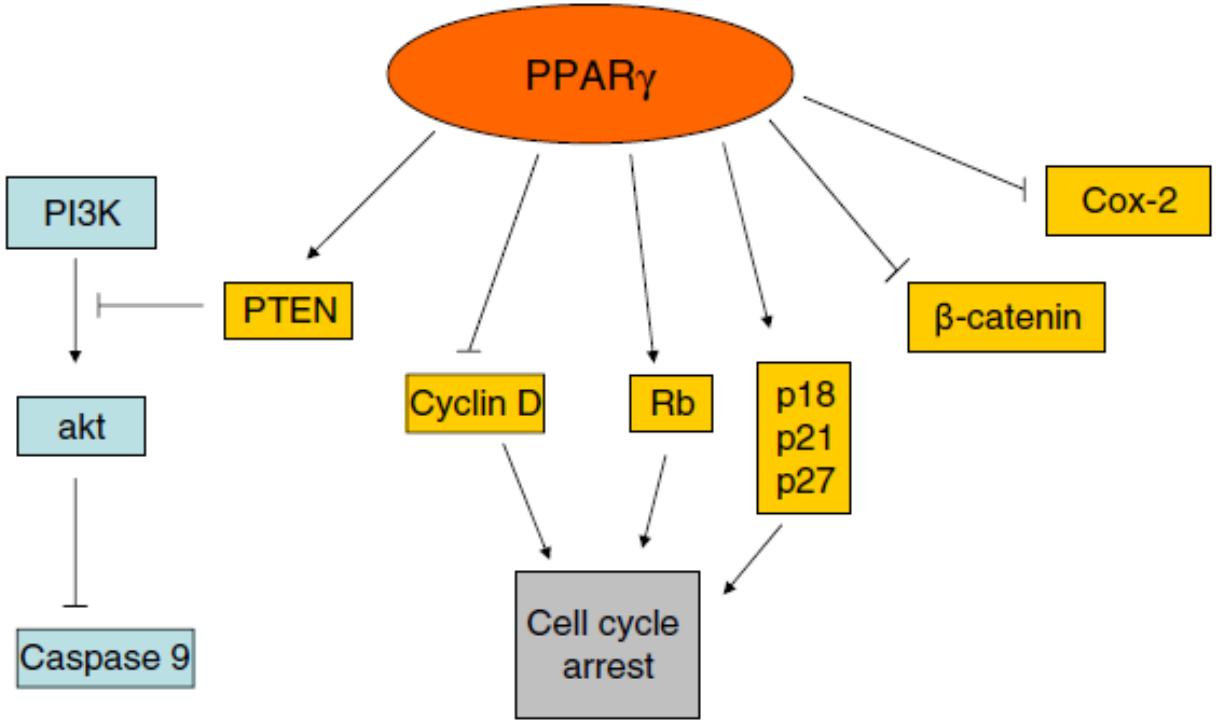
Table 2 | **Effects of PPARγ agonists in various cell types**

Consequence of the treament	Cell type	References
Growth arrest	Human colorectal cancer	118
	Breast cancer	53,54
	Prostate cancer	59
	Myeloid leukaemia	119
	Human neuroblastoma	120
	Human hepatoma	121
	Vascular smooth muscle	122
	Human gastric cancer	123
	Thyroid carcinoma cells	124
	Uterine leiomyoma smooth-muscle cells	125
	Oligodendrocyte-like cells	126
	Pancreatic cell line	68
Cell differentiation	Colon cancer cell lines	45
	Macrophages	127
	Prostate cancer cells	58
	Non-small-cell lung carcinoma	74,128
	Liposarcomas	78,79
	Oligodendrocyte-like cells; rat spinal-cord-derived oligodendrocytes	126
Apoptosis	Human colon cancer cell line	129
	Breast cancer cells	54
	Macrophages	130
	B-lineage cells	131
	Human liver cancer cells	132
	Choriocarcinoma cells	133
	Adipocytes	134
Decrease of spontaneous immortalization	Li–Fraumeni-syndrome-derived human mammary epithelial cells	135

This table summarizes the effects of treatment with PPARγ agonists in various cell types, with regards to cell cycle. In most of the studies, treatment of the cells with a PPARγ agonist has anti-carcinogenic consequences. Most of these results remain to be confirmed *in vivo* and their clinical relevance is not yet proven. Additionally, it is important to note that PPARγ agonists might also have PPARγ-independent effects (see Box 2) that were not separated from the PPARγ-dependent effects in most of the studies.

Michalik L et al., Nature Rev Cancer 2004

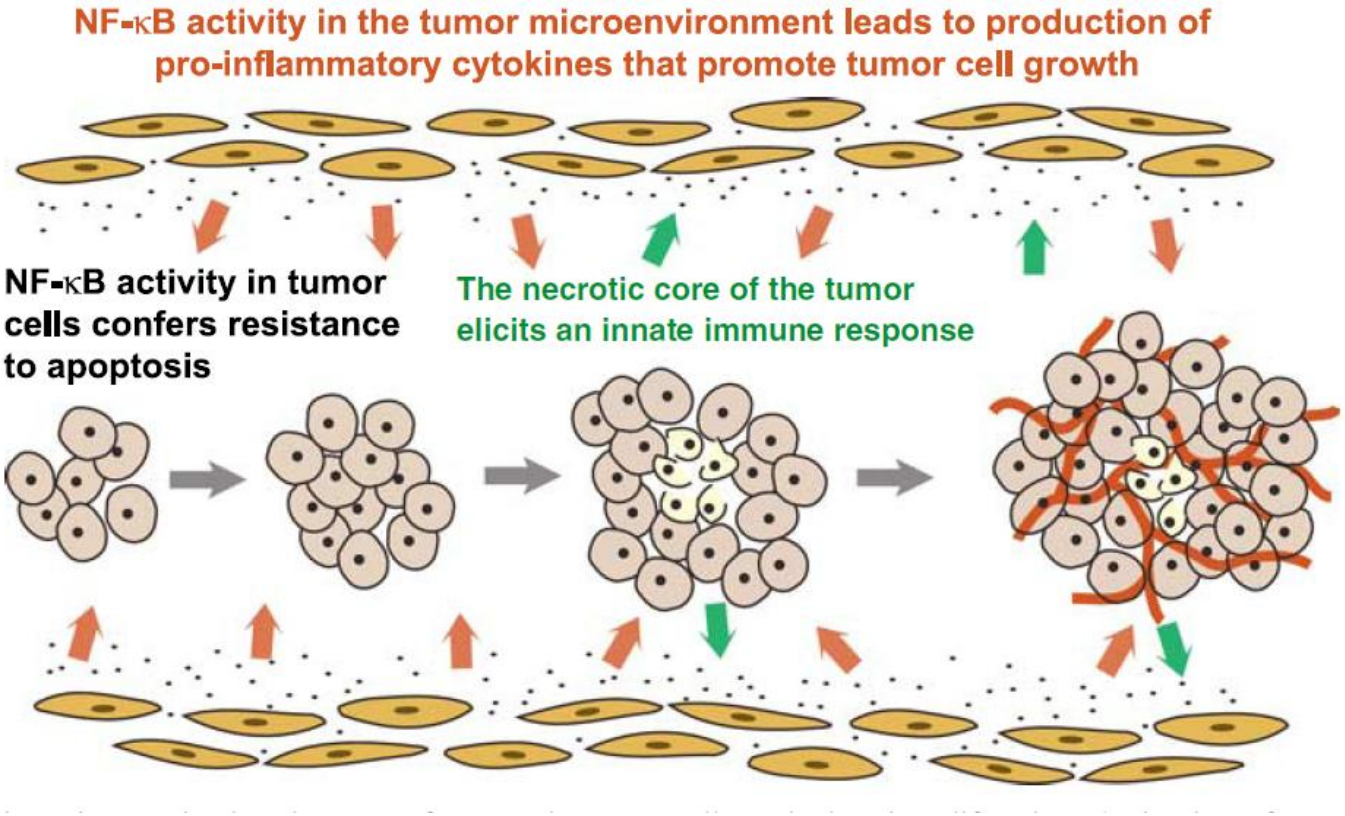
Důležité signální dráhy a molekuly indukované či inhibované PPAR



PPARγ indukuje fosfatázu PTEN vedoucí k inhibici kinázy Akt.
Akt má antiapoptické účinky (inhibice kaspázy-9).
PPARγ způsobuje zástavu bun. cyklu represí cyklinu D, indukcí p18, p21, p27 a interakcí s Rb. PPARγ rovněž potlačuje beta-katenin a COX-2 podporující karcinogenezi kolonu.

Voutsadakis IA, J Cancer Res Clin Oncol 2007, 133:917

Vnitřní a parakrinní účast NFκB v přežívání a proliferaci nádorů.



Aktivace NFκB vede k rezistenci k apoptóze, Buňky na okraji rychle rostoucího nádoru podléhají nekróze, když chybí ATP. Nekrotické nádorové buňky uvolňují prozánětlivé faktory. Tyto faktory aktivují imunitní odpověď nádorového mikroprostředí, která vede k syntéze prozánětlivých cytokinů závislé na NFκB , což podporuje růst nádoru.

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ