DNA Single Strand Break Repair, Neuro-Development, and Brain Tumor

이영수

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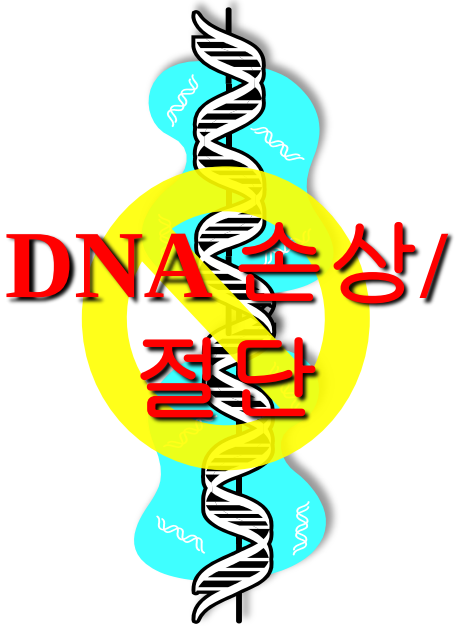
유전체
불안정성



DNA 손상 복구
(DNA damage repair)

세포분열 정지
(cell cycle arrest)

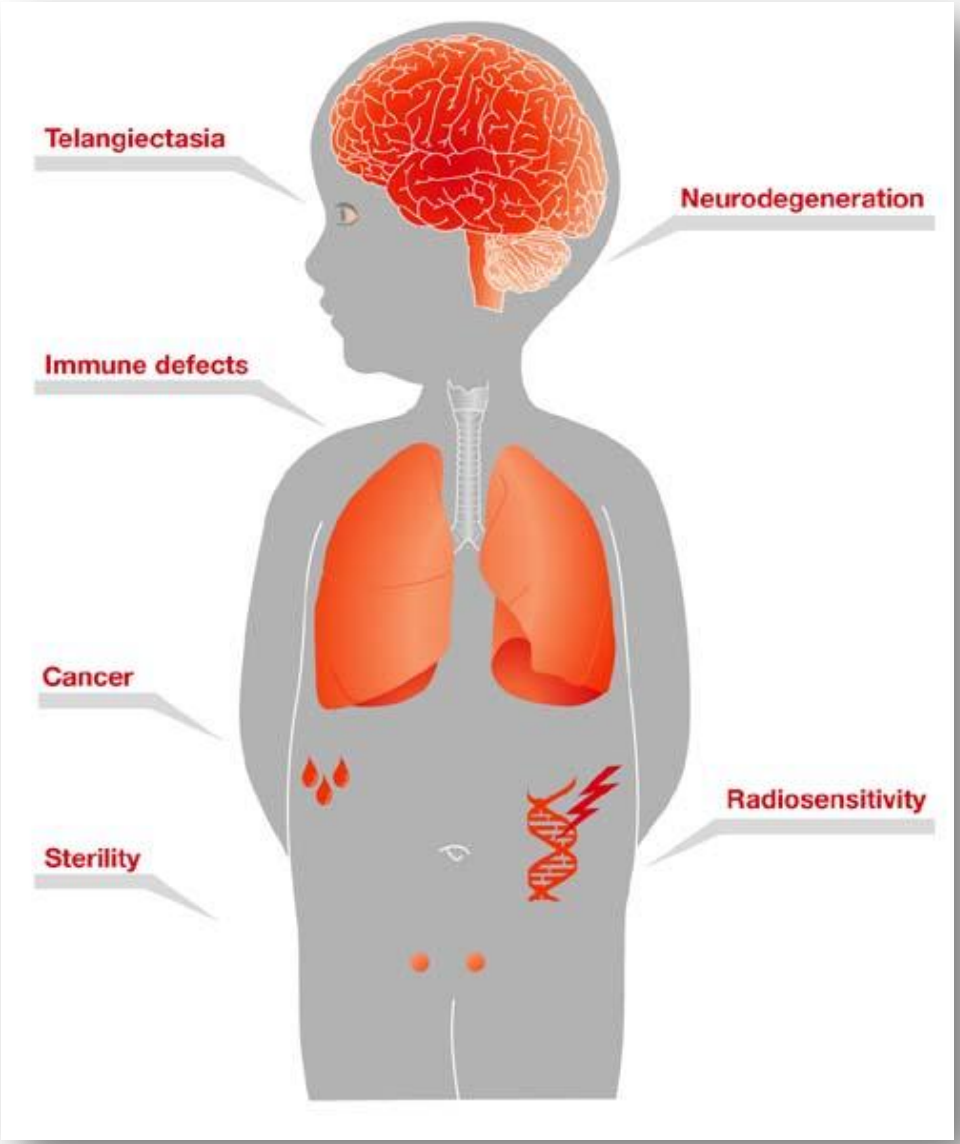
세포사멸
(apoptosis)

드문 유전병과 신경병리

Gene	Syndrome	Neurological features
	DNA DSBs detection/repair related	
ATM	Ataxia telangiectasia (AT)	ataxia, progressive neurodegeneration
ATR	Seckel syndrome (SS)	microcephaly, mental retardation
NBS1	Nijmegen breakage syndrome (NBS)	microcephaly, mental retardation, medulloblastoma
Mre11	AT-like disorder (ATLD)	ataxia, progressive neurodegeneration
DNA ligase 4	LIG4 syndrome	microcephaly
Cernunnos/ XLF	NHEJ1 syndrome	microcephaly
BLM/RecQl3	Bloom syndrome (BS)	mental retardation
FANC/ BRCA2	Fanconi anemia (FA)	mental retardation, medulloblastoma
RecQl4	Rothmund-Thomson Syndrome (RTS)	mental retardation

DNA 이중 나선 절단 관련 유전병

드문 유전병과 신경병리



Ataxia Telangiectasia (A-T)

- **Mutations in the Ataxia telangiectasia mutated (ATM) gene**
- **Autosomal recessive disorder**
- **Progressive cerebellar ataxia**
- **(운동실조)**
- **Oculomotor apraxia**
- **Protein ATM is a protein kinase and responds to DNA double strand breaks (DBSs) – p53 is one of the significant substrates.**

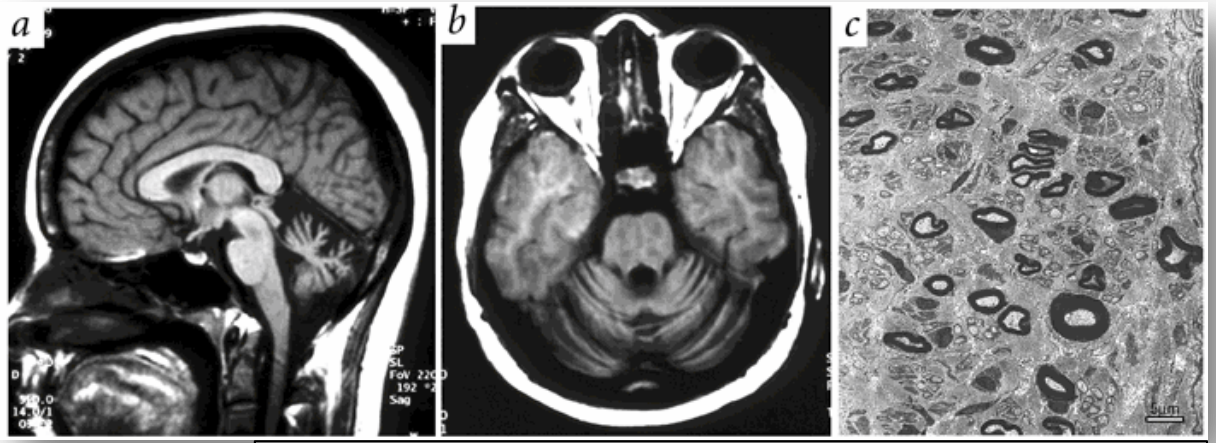
드문 유전병과 신경병리

Gene	Syndrome	Neurological features
	DNA SSBs detection/repair related	
Tyrosyl-DNA phosphodiesterase (TDP1)	Spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), AT like syndrome	cerebellar ataxia, neurodegeneration
Aprataxin (APTX)	Ataxia with ocular motor apraxia 1 (AOA1)	cerebellar ataxia, progressive neurodegeneration, oculomotor apraxia
Senataxin (SETX)	Ataxia with ocular motor apraxia 2 (AOA2)	progressive cerebellar ataxia
Gene unknown (PIK3R5 ?)	Ataxia with ocular motor apraxia 3 (AOA3)	Ataxia, oculomotor apraxia
Polynucleotide kinase Phosphatase (PNKP)	Microcephaly with seizures (MCSZ)	microcephaly, seizure

DNA 단일 나선 절단 관련 유전병

드문 유전병과 신경병리

Spinocerebellar ataxia with axonal neuropathy (SCAN1)



Nature Genetics 2002 Vol. 32(2) pp 267-272

- Saudi Arabian family
- Early onset (13~15 years old), Normal intelligence
- Cerebellar **ataxia** (운동실조 cerebellar atrophy) and peripheral axonal motor and sensory neuropathy (affecting large, terminally differentiated, non-dividing neuronal cells)
- No predisposition to neoplasia or dysfunctions in rapidly replicating tissues
- mutation in the TDP1 **(Tyrosyl-DNA phosphodiesterase 1)** gene
- Repairing stalled Top1-DNA complexes, 3-OH'processing

드문 유전병과 신경병리

Gene	Syndrome	Neurological features
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ATM	Ataxia telangiectasia (AT)	운동실조 (ataxia)
ATR	Seckel syndrome (SS)	
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Mre11	AT-like disorder (ATLD)	뇌신경퇴화 (neurodegeneration)
DNA ligase 4	LIG4 syndrome	
Cernunnos/XLF	NHEJ1 syndrome	
BLM/RecQl3	Bloom syndrome (BS)	소뇌증 (microcephaly)
FANC/BRCA2	Fanconi anemia (FA)	
RecQl4	Rothmund-Thomson Syndrome (RTS)	
	DNA SSBs detection/repair related	
Tyrosyl-DNA phosphodiesterase (TDP1)	Spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), AT like syndrome	정신지체 (mental retardation)
Aprataxin (APTX)	Ataxia with ocular motor apraxia 1 (AOA1)	
Senataxin (SETX)	Ataxia with ocular motor apraxia 2 (AOA2)	뇌암 (brain tumor)
PIK3R5 ?	Ataxia with ocular motor apraxia 3 (AOA3)	
PNKP	Microcephaly with seizure (MCSZ)	

유전체 불안정성

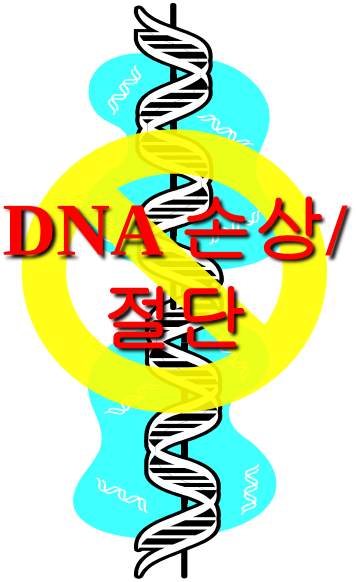
운동실조
(Ataxia)
신경세포 소멸
(Neurodegeneration)

소뇌증
(Microcephaly)
정신지체
(Mental retardation)

뇌암 (Brain Tumor)

DNA 손상복구 기전

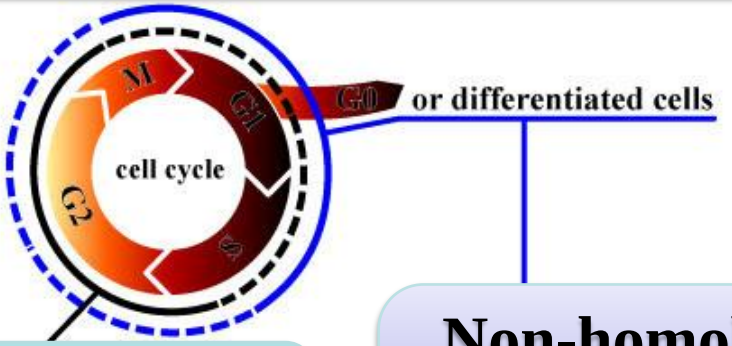
- Excision of damaged, mispaired or incorrect bases
  - Base excision repair (BER)
  - Nucleotide excision repair (NER)
  - Mismatch repair (MMR)



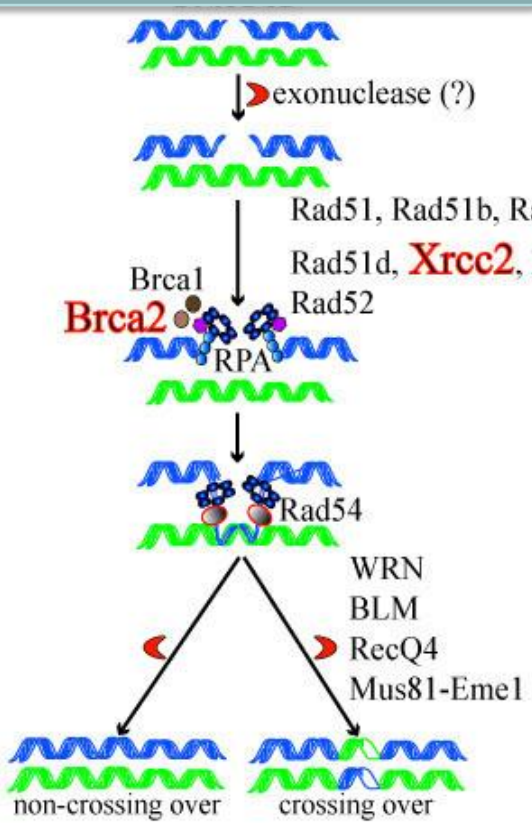
- **Strand break repair**
  - **Single-strand break repair (SSBR)**
  - **Double-strand break repair (DSBR)**
    - ✓**Homologous recombination (HR)**
    - ✓**Non-homologous end joining (NHEJ)**

DNA Double-strand break repair (DSBR)

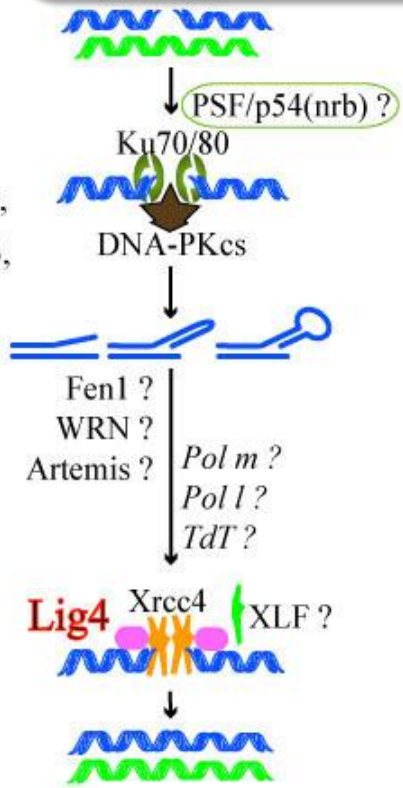
DNA 이중 나선 절단 (DSBR)



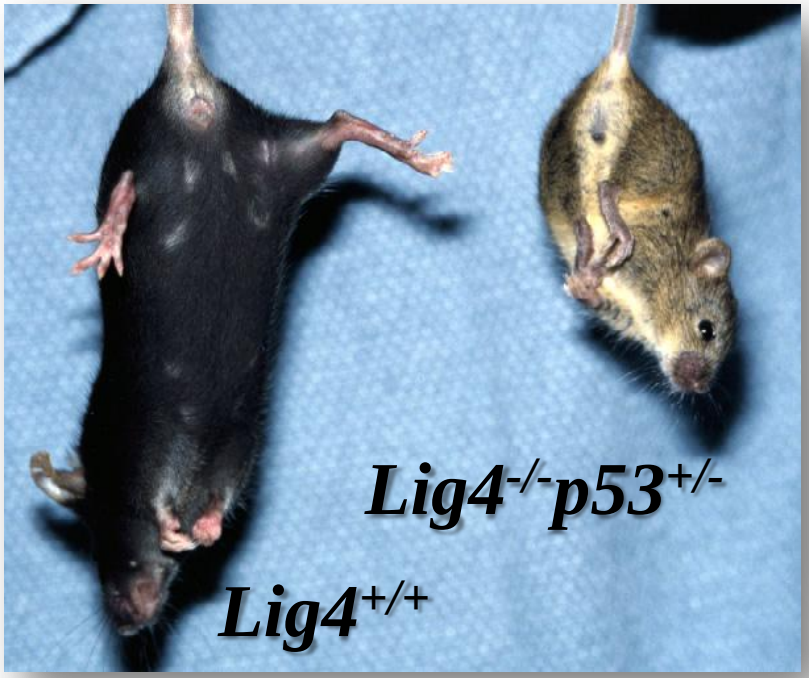
Homologous recombination repair

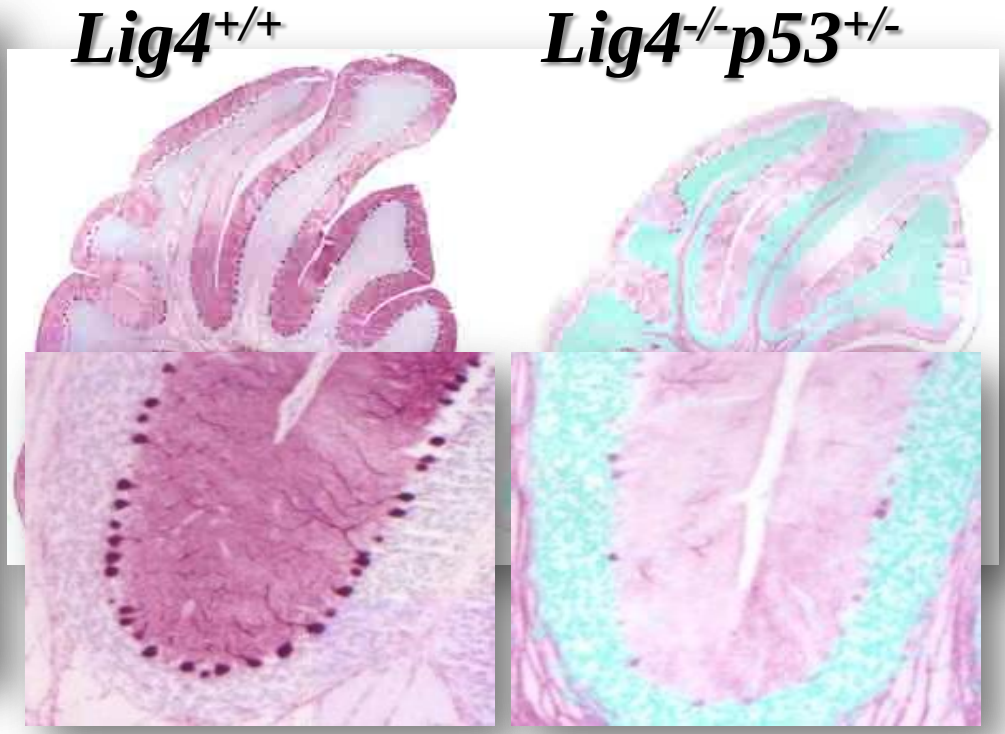


Non-homologous end-joining repair



Lig4 deficiency leads to neurodegeneration in the cerebellum.





Lig4 불활성화에 의한 유전체불안정성이 소뇌 Purkinje 세포의 소멸을 유도한다.

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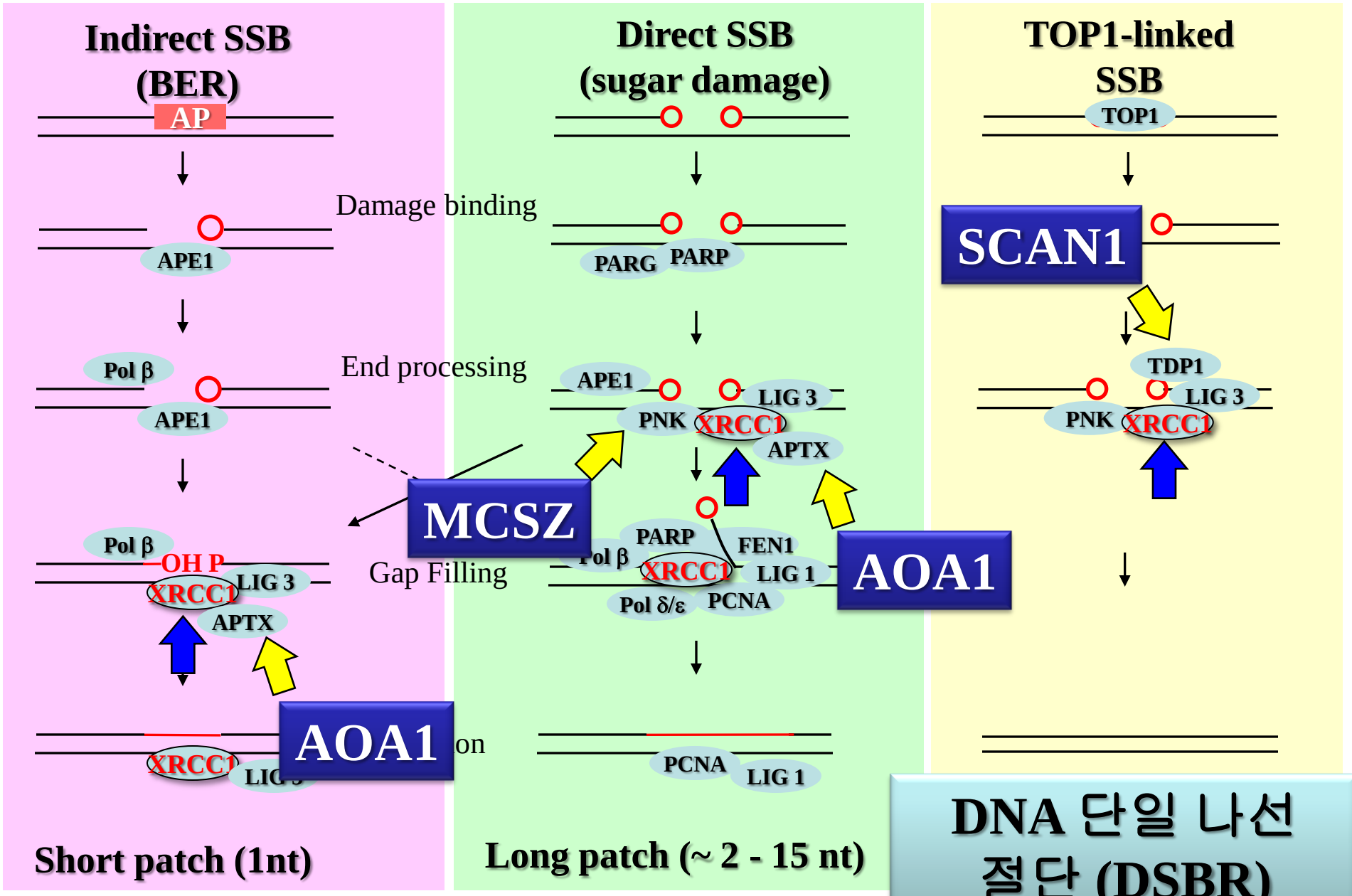
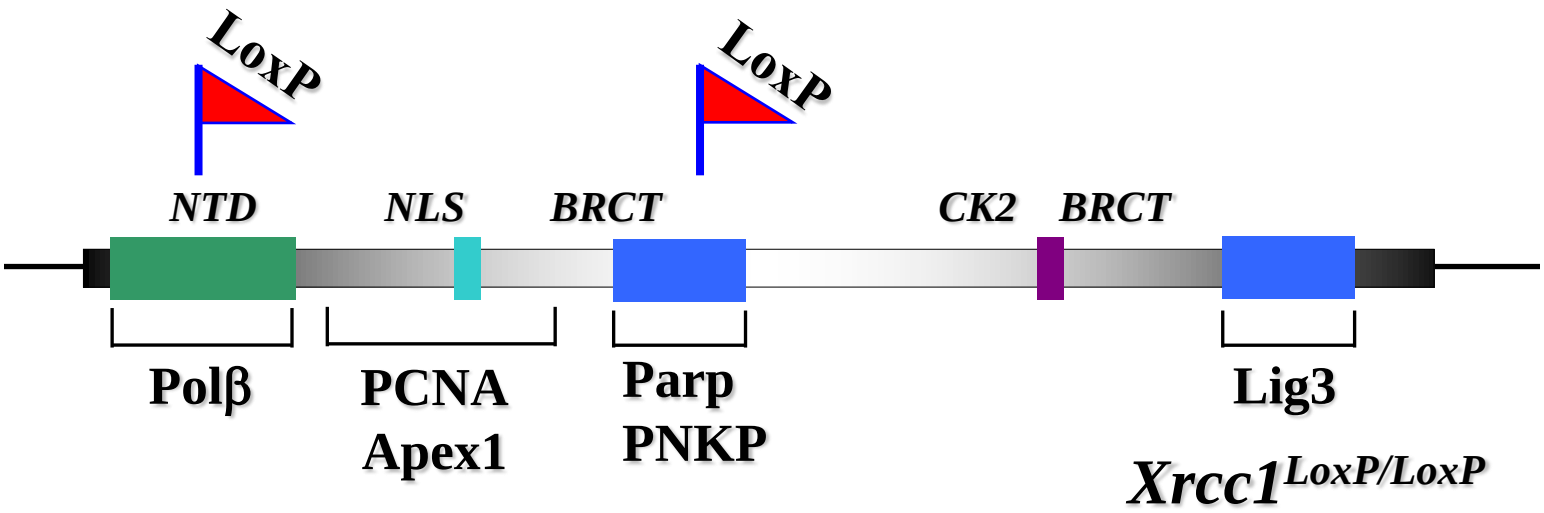
DNA Single-strand break repair (SSBR)



Xrcc1



SSB repair defect - *Xrcc1* 불활성화; 배아형성중 치사~E9

SSB repair defect - *Xrcc1*$^{LoxP/LoxP}$; *NestinCre* (*Xrcc1*$^{Nes\text{-}Cre}$)

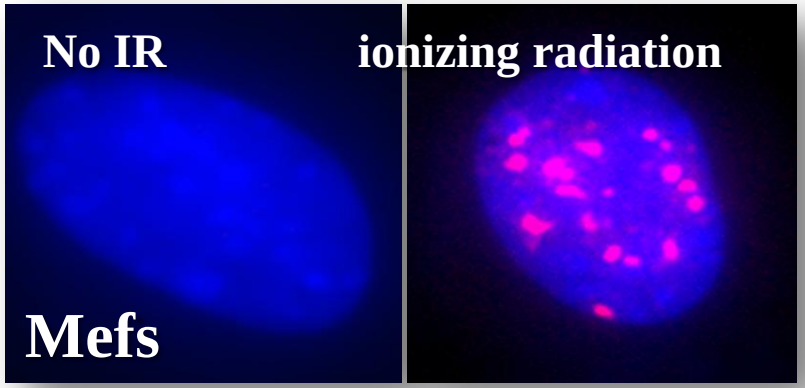
; 신경계에서만 선택적 불활성화

No embryonic lethality (배아 치사) and

***Xrcc1*$^{Nes\text{-}Cre}$ 뇌에서 특별한 이상 발견되지 않음**

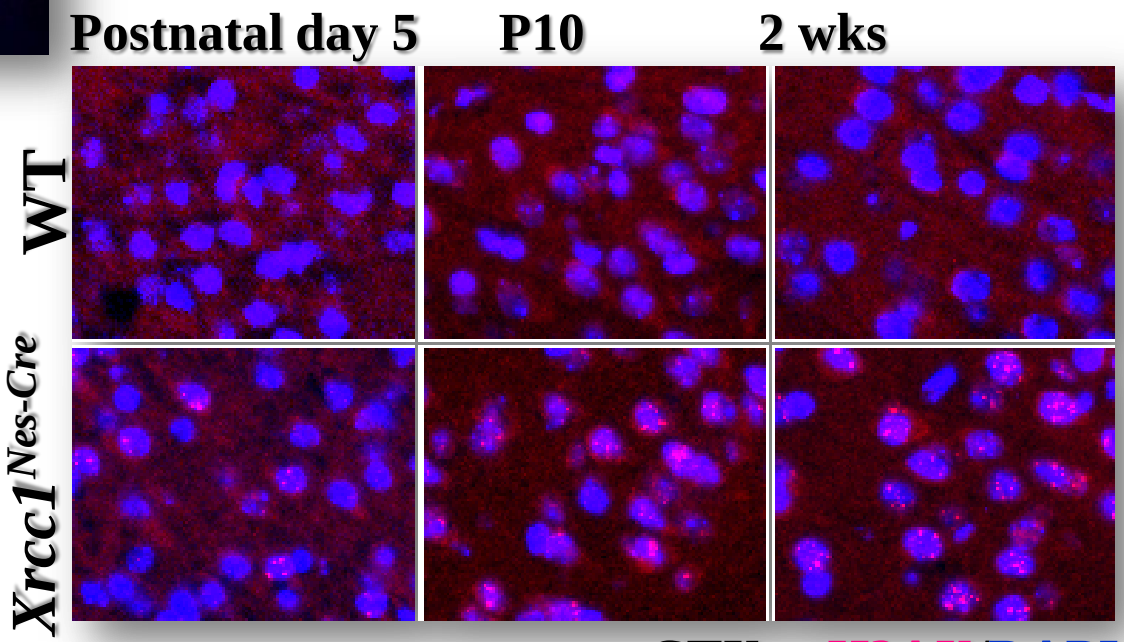
DNA 손상이 H2AX 인산화 유도 (g-H2AX).

γ-H2AX foci/DAPI



H2AX is a member of histone H2A family.

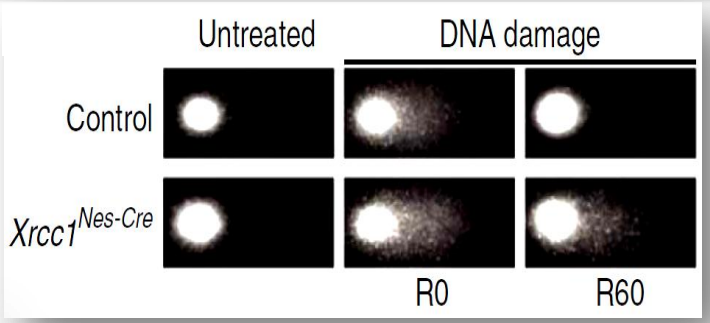
Rapid phosphorylation of serine 139 of H2AX upon DNA damage

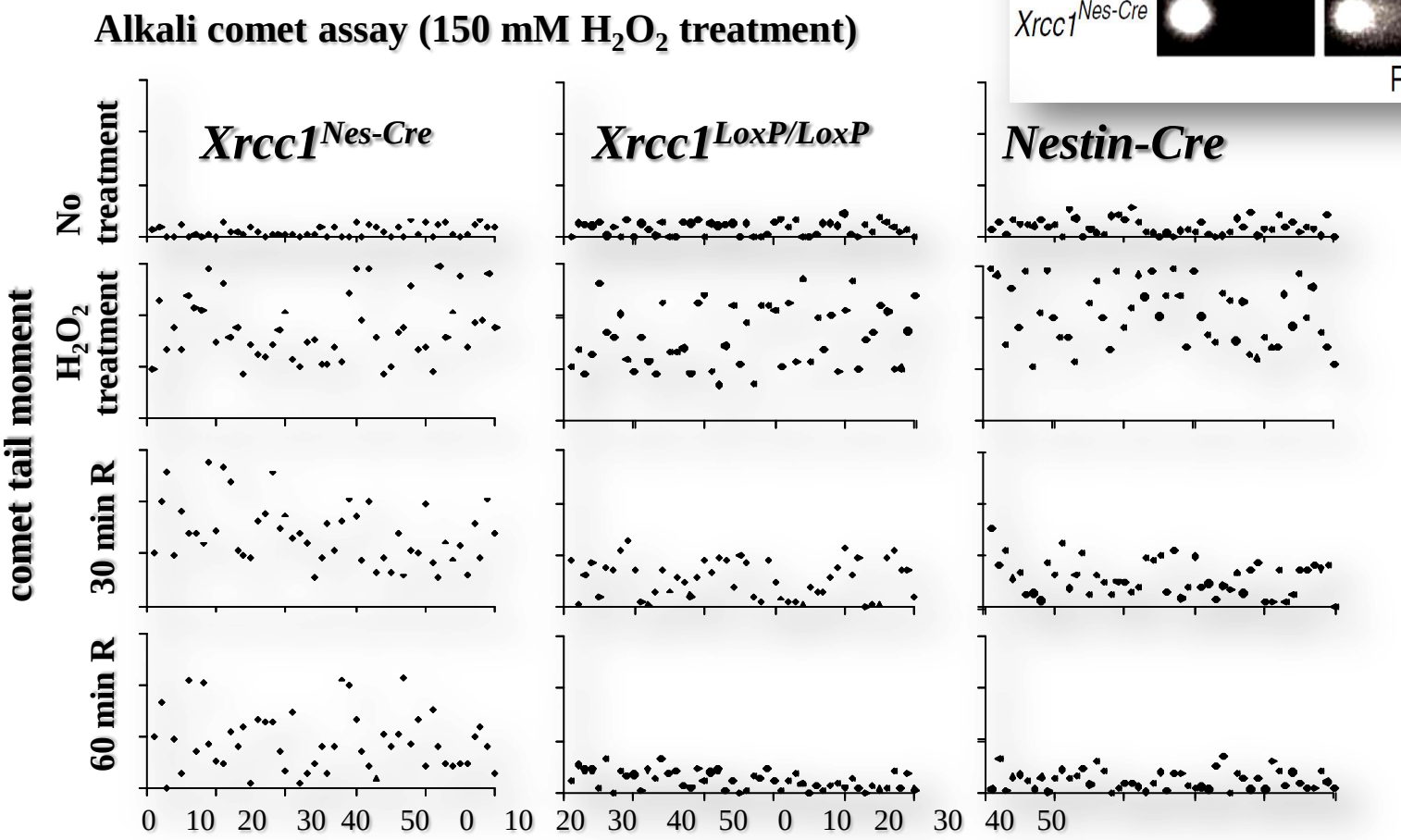


CTX, γ-H2AX/DAPI

성숙된 신경세포에 DNA 손상이 지속적으로 생성 및 축적

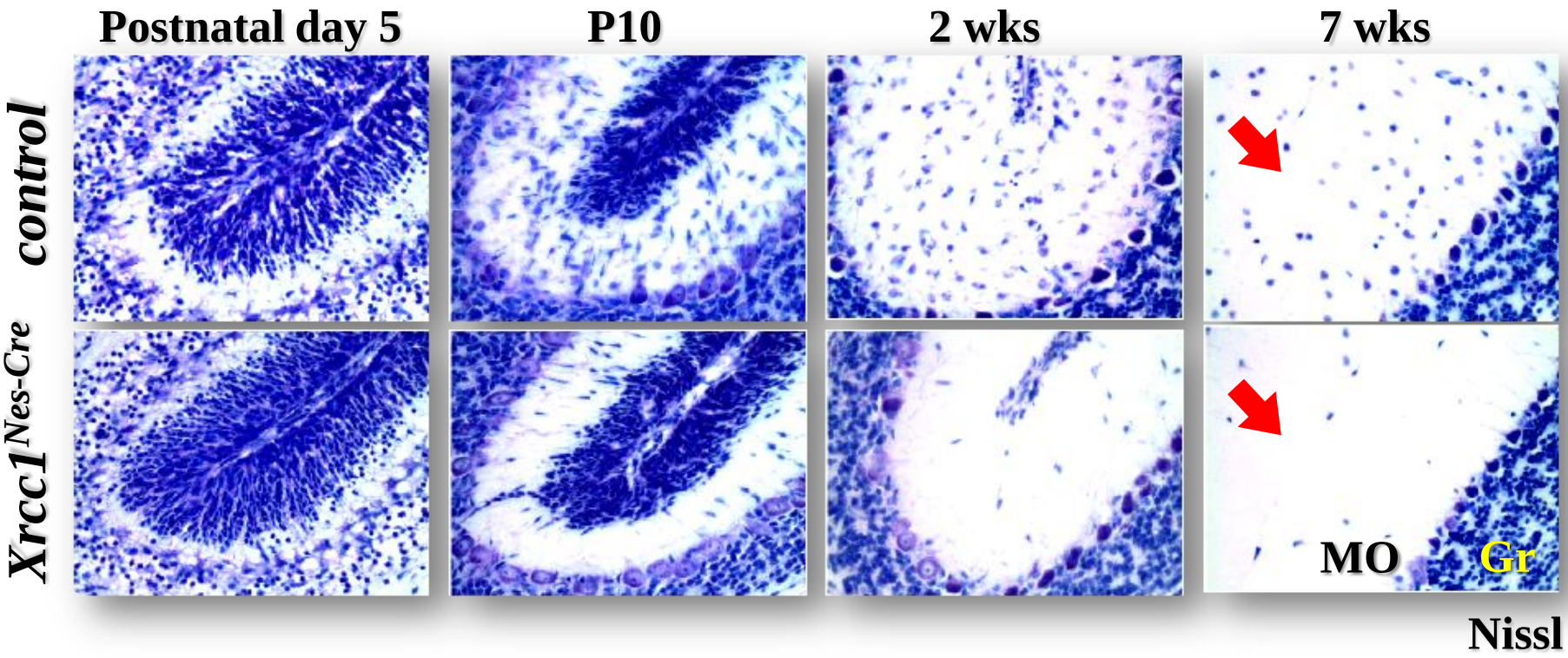
Defective DNA damage repair in astrocytes





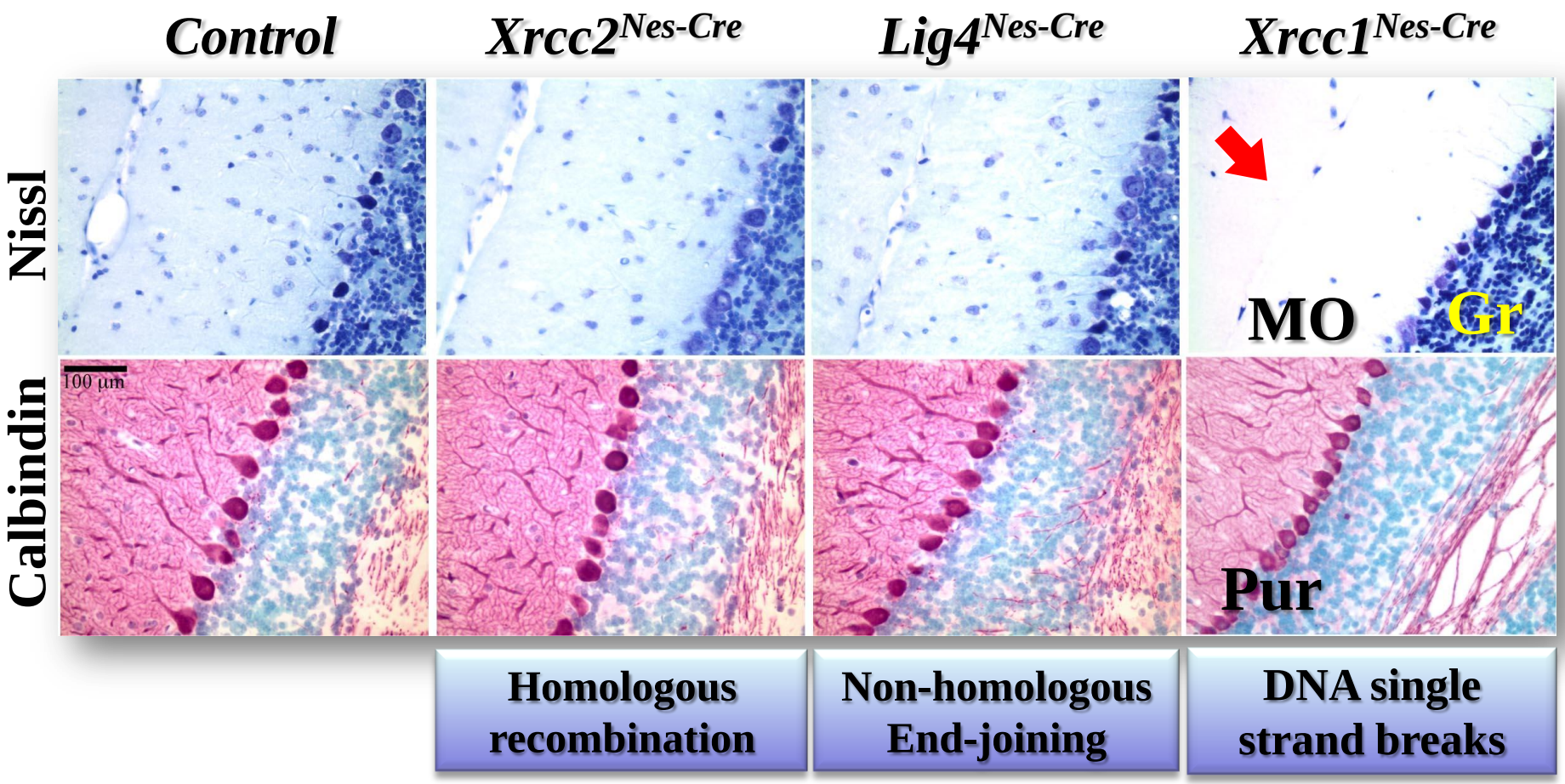
Xrcc1 불활성화가 astrocyte에서 DNA 손상 복구능력을 저해한다.

Xrcc1$^{Nes\text{-}Cre}$



Xrcc1 불활성화에 의한 유전체불안정성이 소뇌의 interneuron 형성을 저해한다.

Xrcc1$^{Nes\text{-}Cre}$



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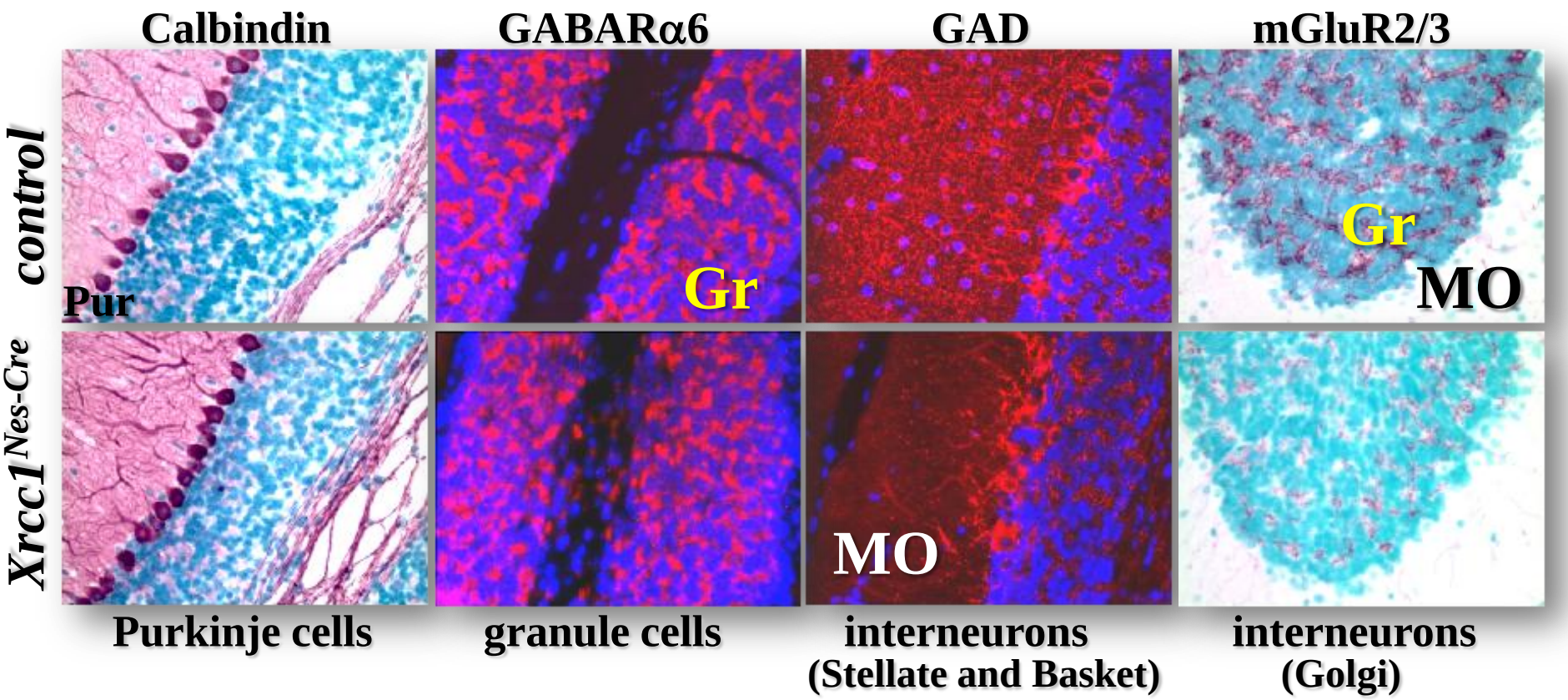
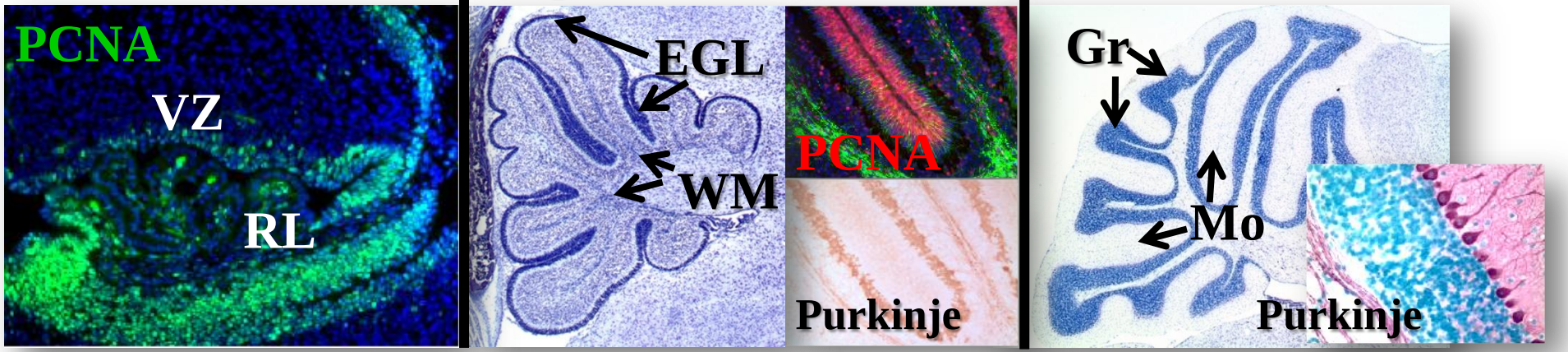
Xrcc1$^{Nes\text{-}Cre}$



Xrcc1 불활성화에 의한 유전체불안정성이 소뇌의 interneuron 형성을 저해한다.

소뇌 발생

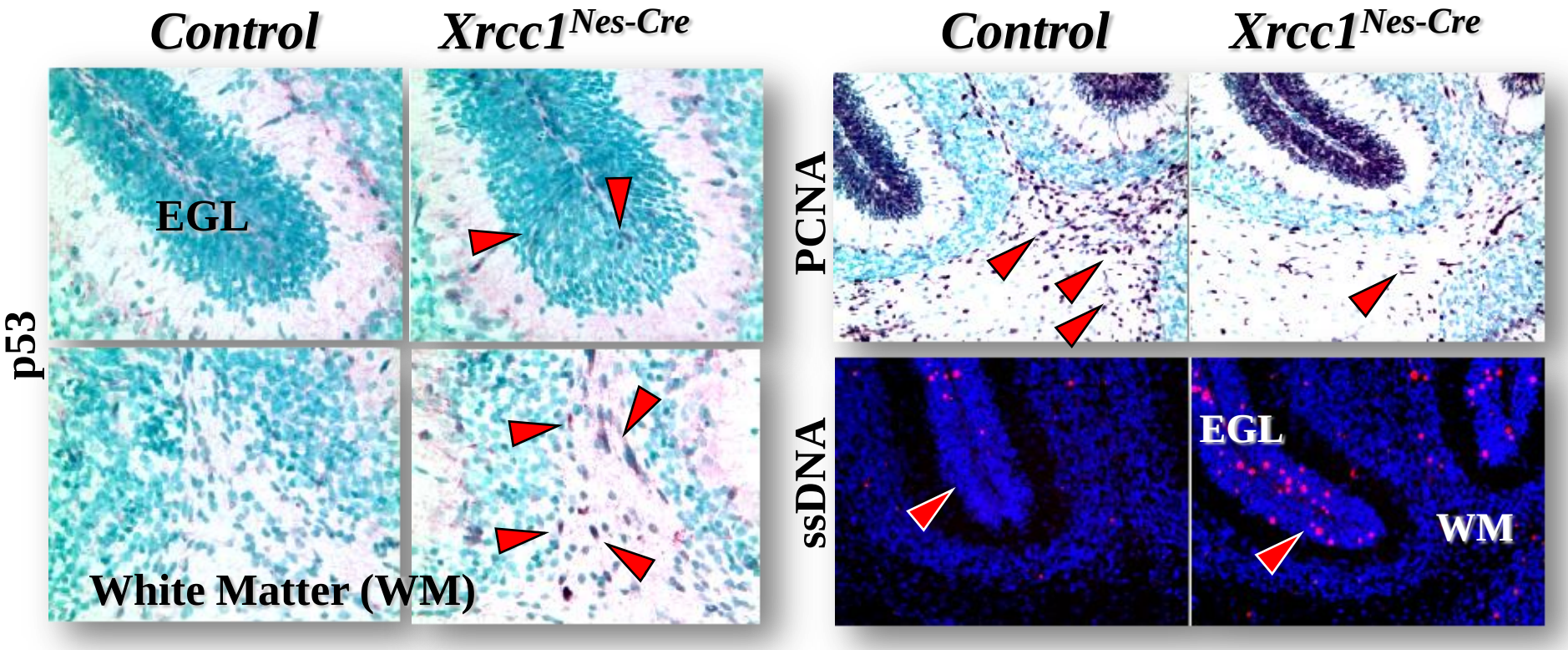
embryonic | **postnatal (~P14)** | **mature**



Rhombic lip (RL) ⇨ **External germinal layer (EGL)** ⇨ **Granule cell layer (Gr)**
Granule cell progenitor

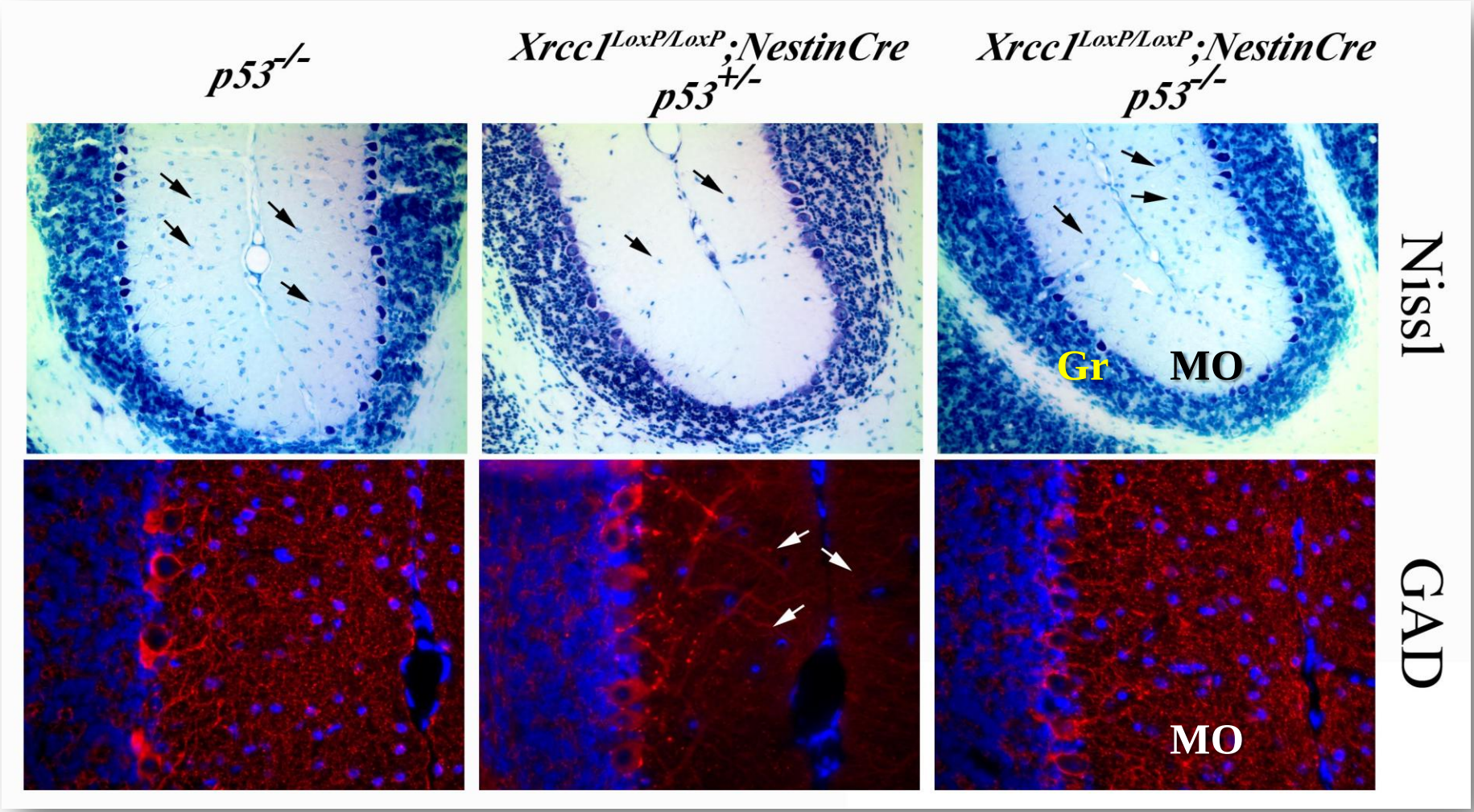
Ventricular zone (VZ) Progenitor ⇨ **Purkinje cell layer**
White matter (WM) Progenitor ⇨ **Cerebellar Interneurons (Basket/ Stellate/ Golgi)**

Xrcc1$^{Nes\text{-}Cre}$



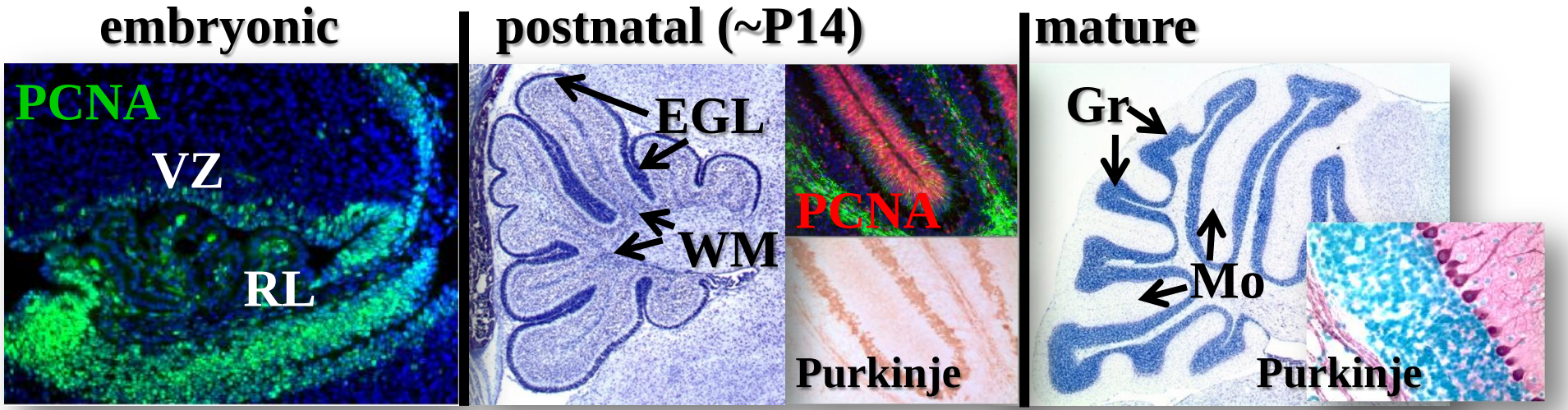
Xrcc1 불활성화는 소뇌 발생과정에서 white matter 에서는 세포 분열 정지 (cell cycle arrest) 그러나 EGL에서는 세포 사멸 (apoptosis) 을 일으키는데, 이 과정은 p53 (tumor suppressor) 가 관여한다.

$Xrcc1^{Nes\text{-}Cre}p53^{-/-}$



P53 불활성화에 의해서 신경계, 특히 소뇌 이상이 복구 된다.

소뇌 발생



Rhombic lip (RL) ⇨ External germinal layer (EGL) ⇨ Granule cell layer (Gr)
Granule cell progenitor

Ventricular zone (VZ) Progenitor ⇨ Purkinje cell layer
White matter (WM) Progenitor

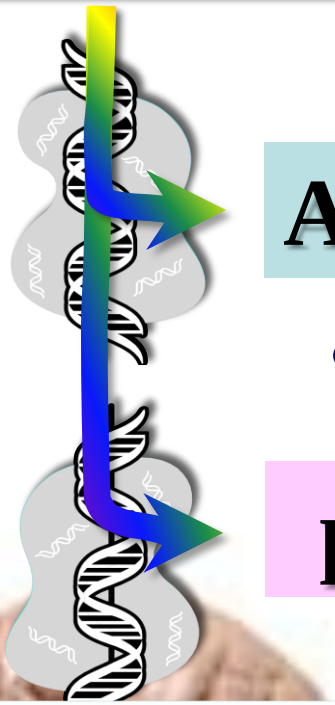
p53 dependent Apoptosis (세포사멸)

p53 dependent cell cycle arrest (세포분열 정지)

Genomic instability resulting from *Xrcc1* deficiency (*NestinCre*)

Nature Neuroscience 12(8):973

Xrcc1
불활성화



ATM

P

p53

DNA 손상 복구
(DNA damage repair)

세포분열 정지
(cell cycle arrest)

세포사멸
(apoptosis)

DNA 손상을 인식한 ATM이
p53를 인산화시켜
DNA 손상 신호전달체계를
작동시킨다.

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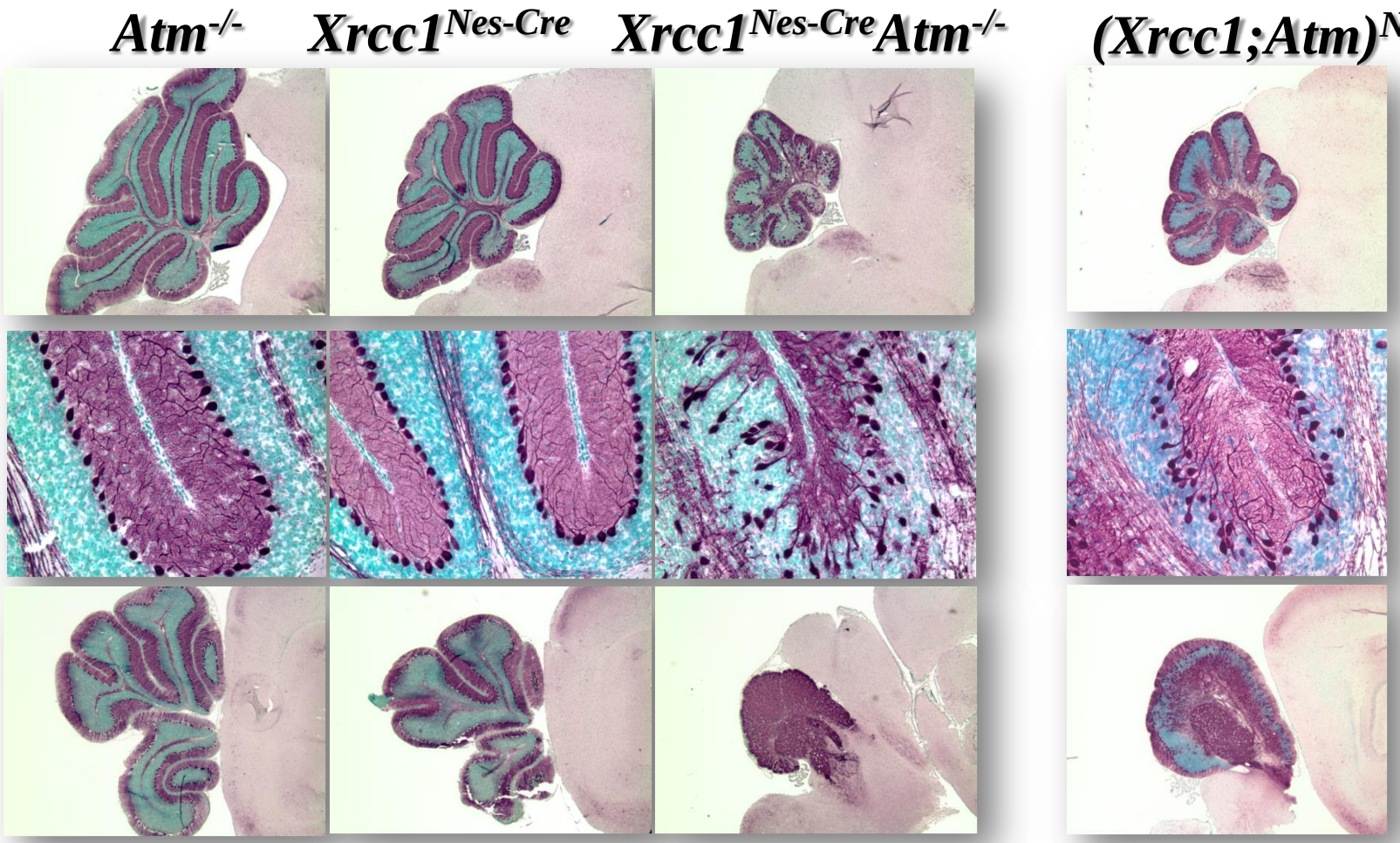
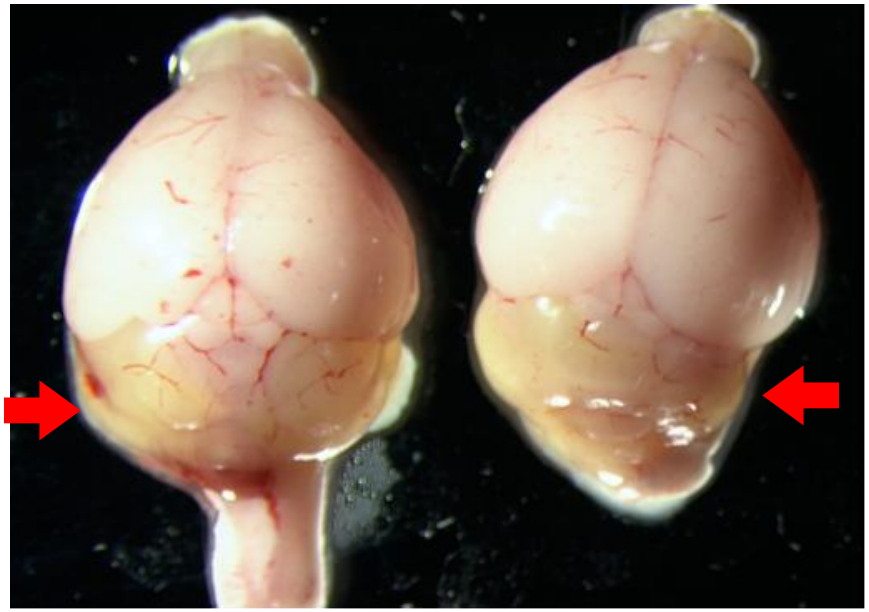
Xrcc1$^{Nes\text{-}Cre}$;Atm$^{-/-}$ or (Xrcc1;Atm)$^{Nes\text{-}Cre}$



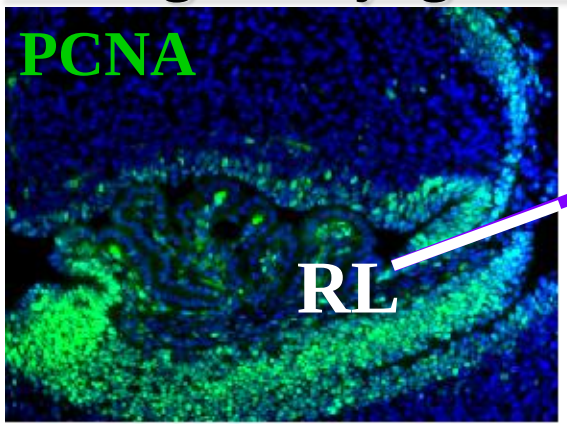
ATM 불활성화는 소뇌의 Purkinje 세포의 배열에 영향을 미친다.

유전체 불안정성이 발암을 유도한다.



medulloblastoma

During embryogenesis



EGL

Granule cells

During postnatal development

p53 dependent apoptosis

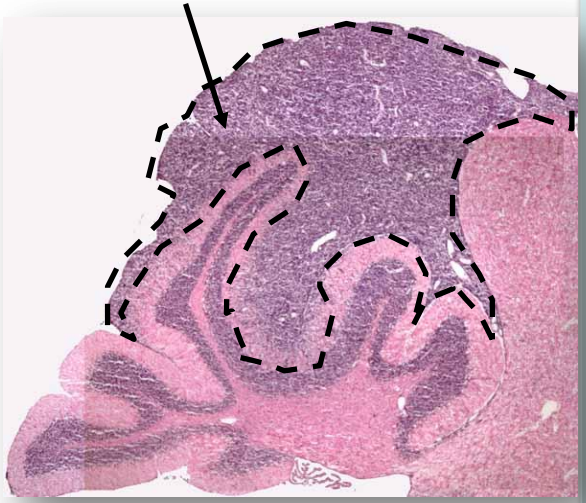
유전체불안정성

뇌암 (Brain tumor)

유전체 불안정성이 발암을 유도한다.

Medulloblastoma (embryonal brain tumor)

BRAIN TUMOR
medulloblastoma



Lig4$^{-/-}$*p53*$^{-/-}$

Cancer Research 63(17):5428

- **Fast-growing, high grade tumor**
- **Relatively rare in all primary brain tumors (2%);most common -> glioma/GBM ~45%**
- **But the most common pediatric brain tumor (18~25%)**
- **Current treatments; surgical removal (as much as possible), and radiation (followed by chemotherapy - older kids/adults)**
- **Five year survival; 50~80%,**
- **However !! , Quality of Life ???**

신경계의 유전체 불안정성은 **Medulloblastoma**의 형성을 유발시킨다.
(~ 100 %)

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유전체불안정성 제어 연구센터

유전체 불안정성에 기인한 **medulloblastoma** 특이 유전자 발현이 유전적 배경에 관계없이 유사하다.

WT adult **$p53^{-/-}$ adult**

Realtime PCR

mRNA Microarray – 유전자의 발현 정도 측정

발현되는 모든 유전자를 동시에 측정 가능

$Brca2^{Nes\text{-}Cre}$

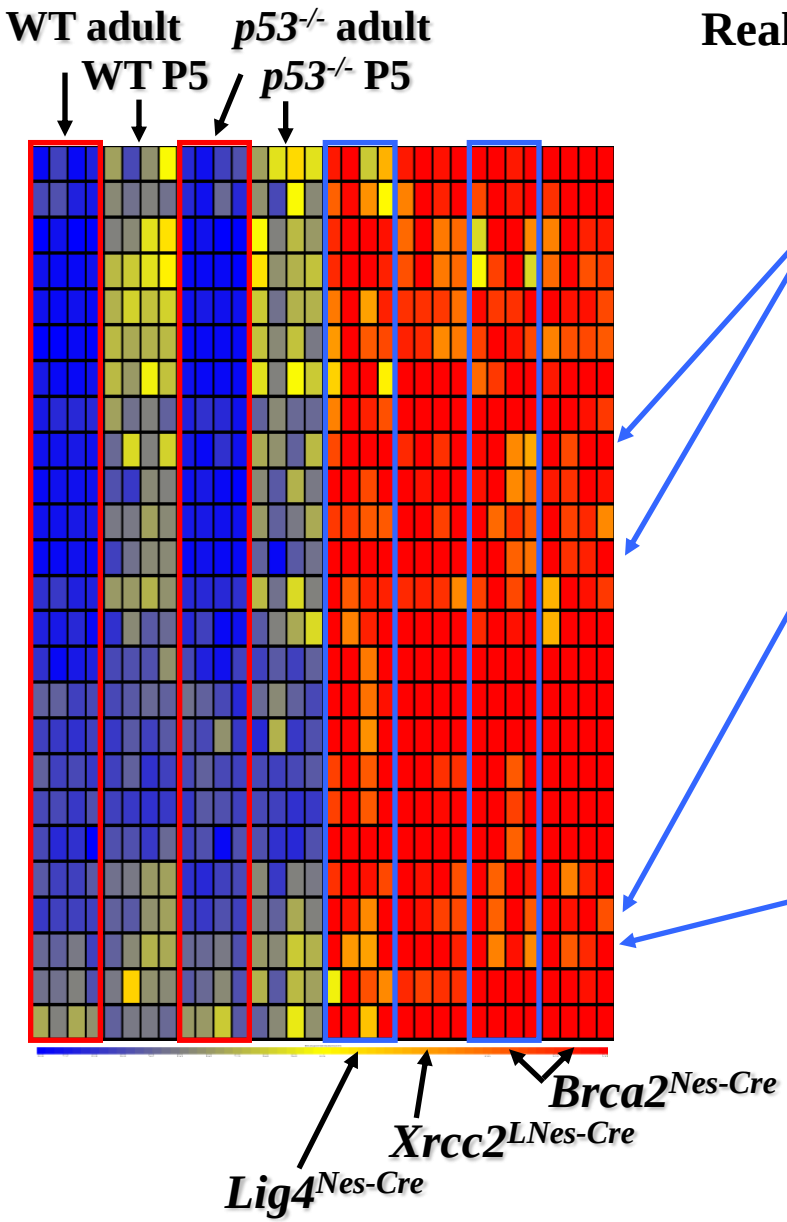
$Xrcc2^{LNes\text{-}Cre}$

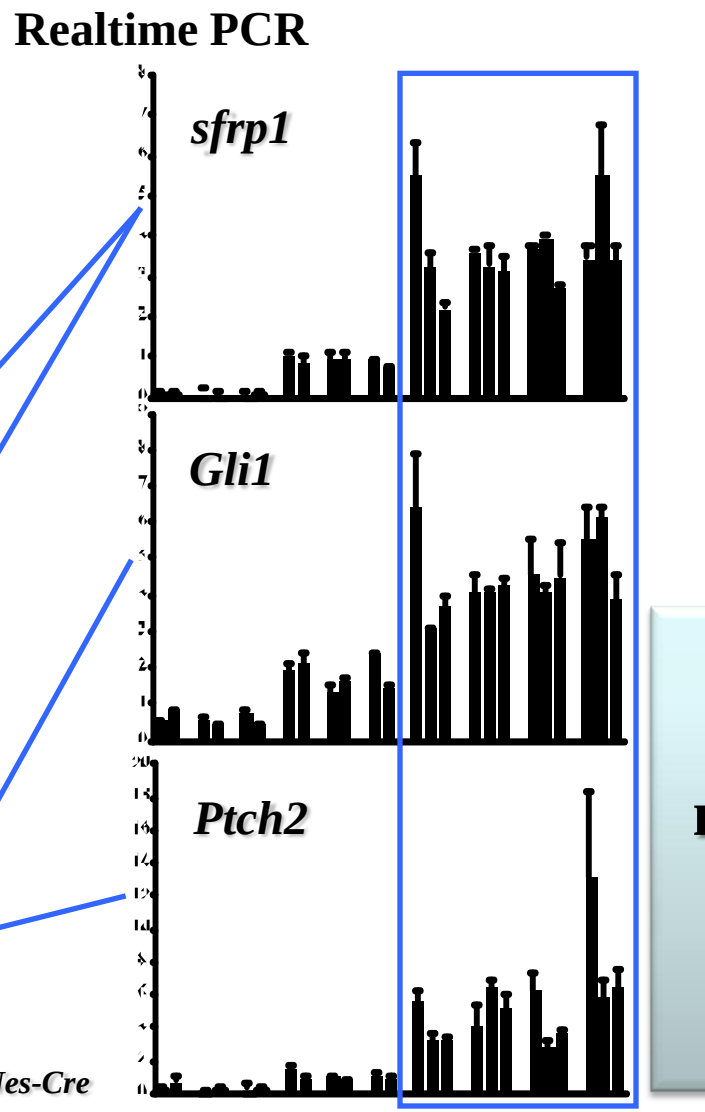
$Lig4^{Nes\text{-}Cre}$

medulloblastoma

AJOU GIRC Genomic Instability Research Center
유전체불안정성 제어 연구센터

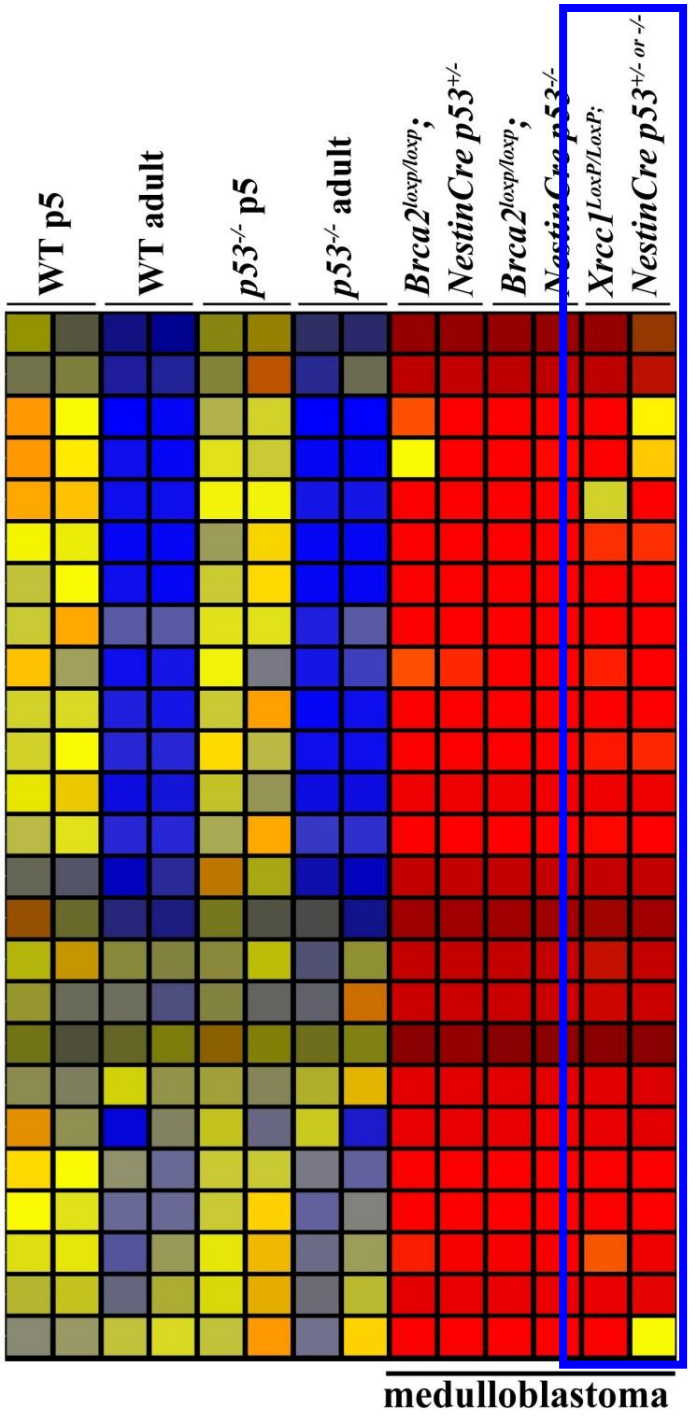
유전체 불안정성에 기인한 medulloblastoma 특이 유전자 발현이 유전적 배경에 관계없이 유사하다.





또한 patched1 변이에 의한 medulloblastoma 의 mRNA 변화와도 비슷하다.

Xrcc1$^{Nes\text{-}Cre}$;*p53*$^{-/-}$ medulloblastoma
Microarray - mRNA profiling



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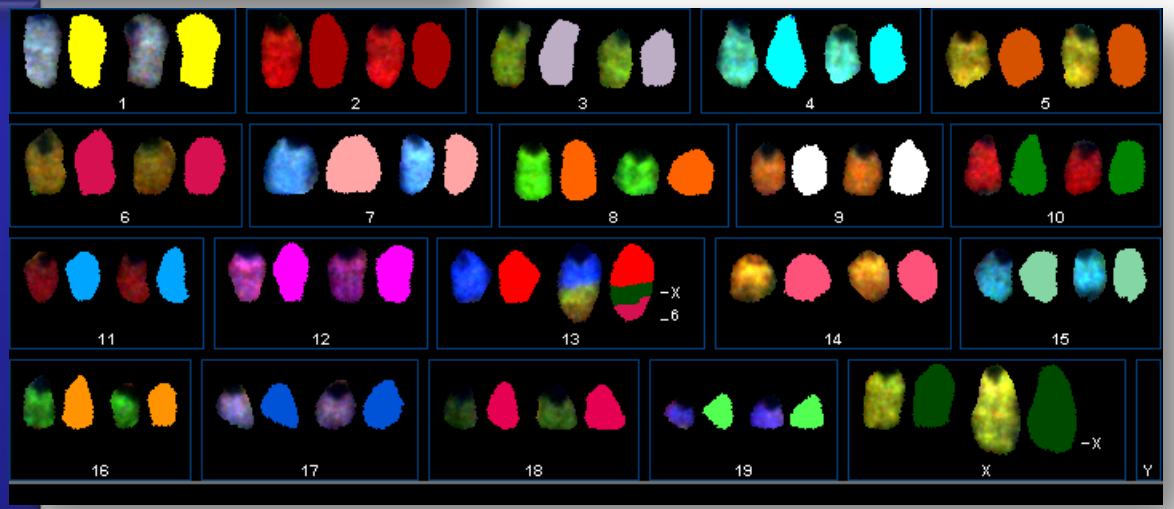
1416759	NM_013815	Mical1 *
1455956_x	AV310588	Ccnd2 *
1430127_a	AK007904	Ccnd2 *
1448710	D87747	Cxcr4
1448650_a	NM_011132	Pole
1439622	AV291679	Rassf4
1449822	BC010820	Math1
1448395	NM_013834	Sfrp1 *
1435338	AW553415	Cdk6
1417155	BC005453	N-myc
1416594	BC024495	Sfrp1 *
1417420	BB538325	Ccdn1
1436996_x	AV066625	Lyzs
1450281_a	NM_023064	Titest *
1427130_x	AJ249392	Titest *
1425957_x	AF257503	Titest *
1416779	BE197945	Sdpr *
1416778	NM_138741	Sdpr *
1443832_s	AV064339	Sdpr *
1449135	NM_009236	Sox18
1449058	NM_010296	Gli1
1422655	NM_008958	Ptch2
1421265_a	NM_019547	Seb4
1422612	NM_013820	HKII

유전체 불안정성에 기인한 medulloblastoma 특이 유전자 발현이 유전적 배경에 관계없이 유사하다.

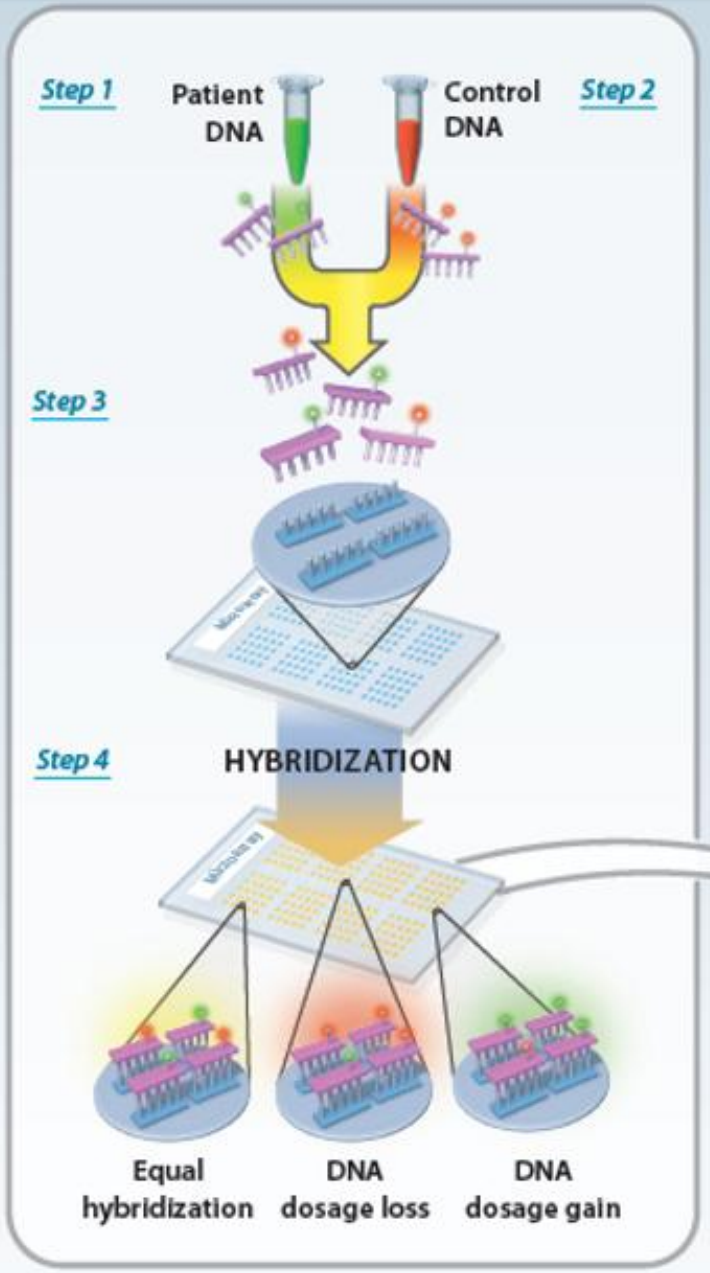
SKY;
Spectral Karyotyping

39,XY,der(13)t(13;?),-10
39,XY,der(13)t(13;?),-10,dup(1),-12,der(18)t(12;18)
39,XY,der(13)t(13;?),-10,-5?12,der(14)t(14;?)
38,XY,der(13)t(13;16),-10dup(4),dep(6)
39,X,der(X)t(X;6)der(11)t(3,11,19),-13,-Y+mar
39,X,der(X)t(X;6)der(11)t(3,11,19),
der(12)t(12;16)-13,der(14)t(Y;14)-16+mar
39,X,der(X)t(X;6)der(11)t(3,11,19),
-13,-Y-11,-12+mar
39,X,der(X)t(X;6)der(11)t(3,11,19),-13,-12,
-14,?16,fusion of 6 and 18-Y+mar
35,XY,-13,
39,XY,der(12)t(7;12),-16
40,XY,der(13)t(6,13)
39,XY,der(12)t(10;12)-8,-10,del(13),
-19,mar(?1,?13,6)
38,XY,der(13)t(8;?10.13),-10,-8



Xrcc2 deficient medulloblastoma
PCNA 103(26):10017

유전체 불안정성에 의한 medulloblastoma는 Patched1 유전자 이상이 원인이다.



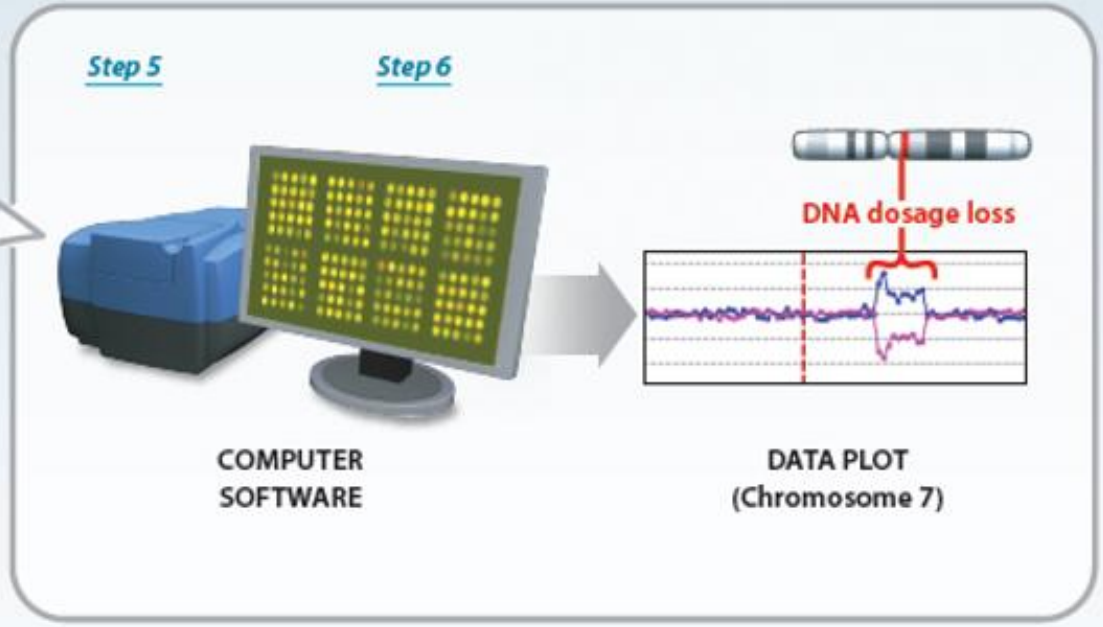
Array CGH: The Complete Process

Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

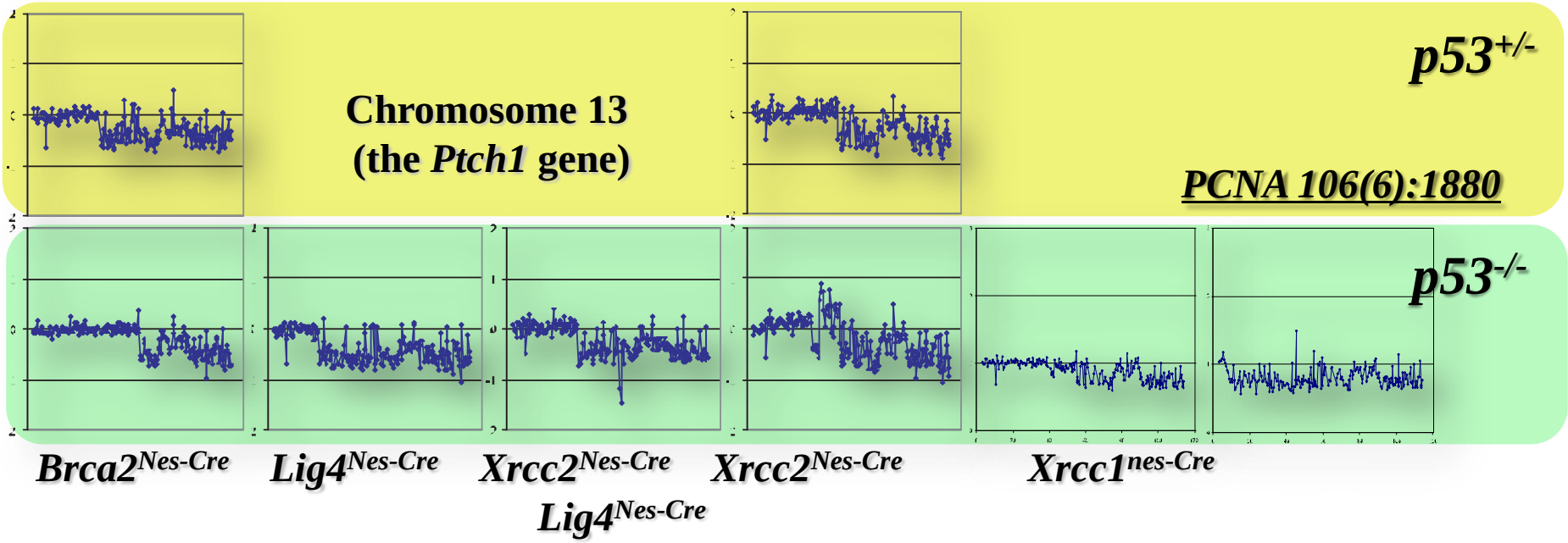
Step 5 The microarray scanner measures the fluorescent signals.

Step 6 Computer software analyzes the data and generates a plot.



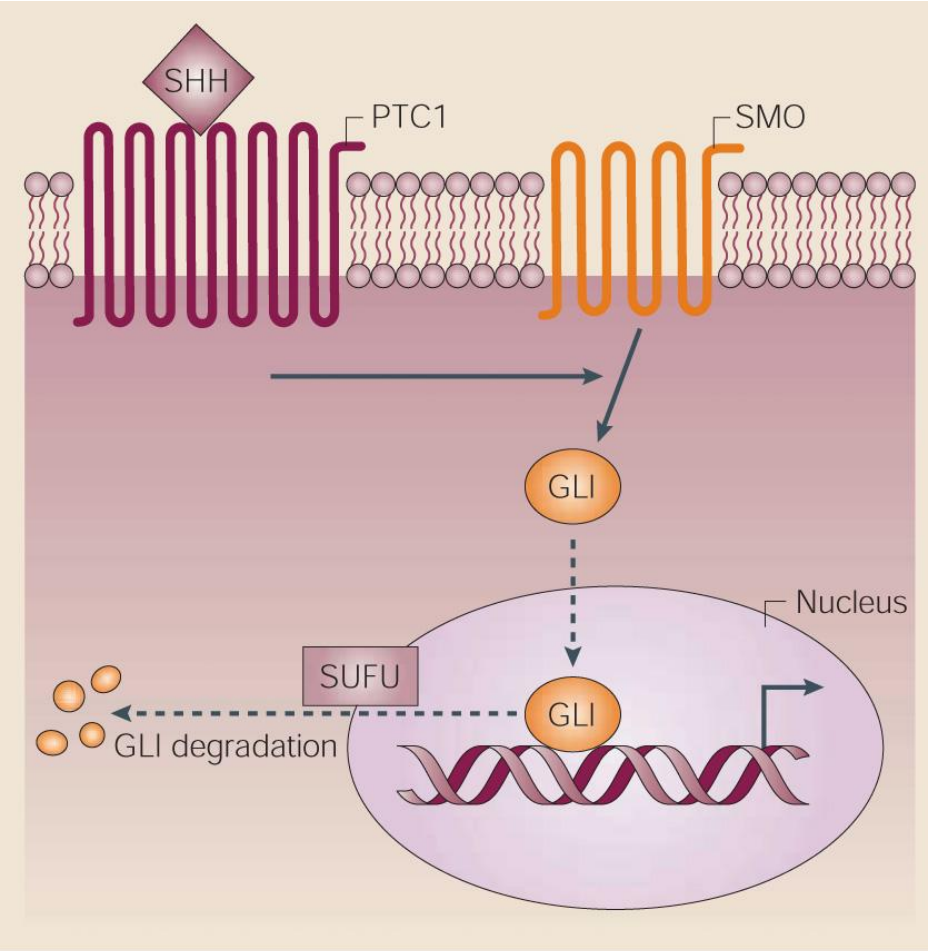
유전체 불안정성에 의한 medulloblastoma는 Patched1 유전자 이상이 원인이다.

Array CGH (comparative genomic hybridization) – Genomic DNA



- **Deletion or loss of the *Patched1* (*Ptch1*) gene in medulloblastoma of DNA repair defective animal models at the genomic level.**
- **Loss of the *Ptch1* gene in the DNA damage repair defective cerebellum is likely a driving force to induce aberrant SHH signaling, leading to medulloblastoma formation.**

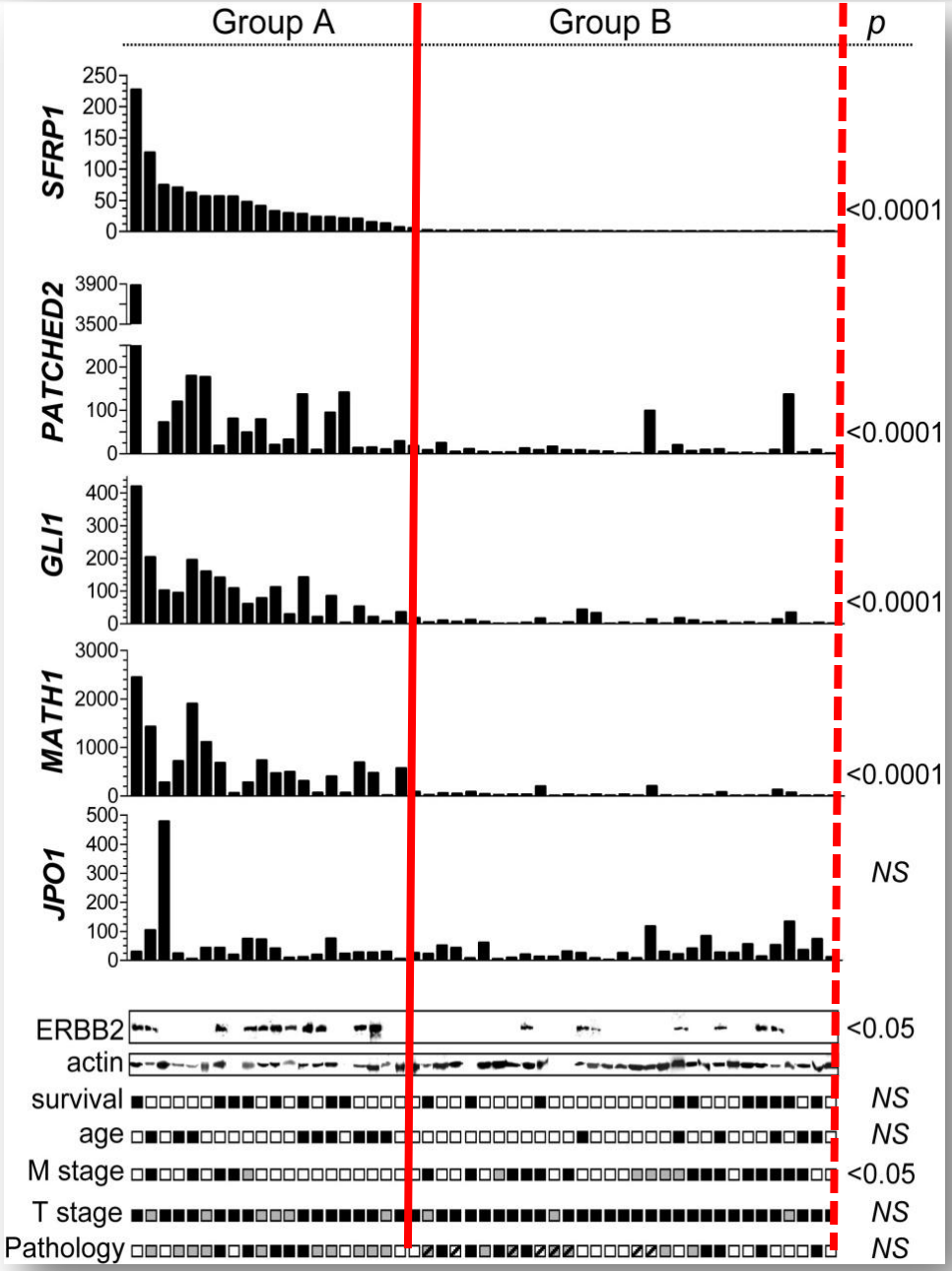
Sonic Hedgehog (SHH) signaling pathway



- **Mutations in the *PTCH1* gene cause Gorlin syndrome (also known as basal cell nevus syndrome).**
- **Clinical features of this syndrome include basal cell carcinoma and medulloblastoma.**

Suzanne Baker and Peter McKinnon, Nature Review Cancer, 2004, vol. 4 (3), pp 184-96

medulloblastoma specific gene expression



- A group of medulloblastoma patients with poor prognosis showed high expression levels of these molecular finger print genes.
- ~ 20% of human medulloblastoma with SHH signalling defects indicative of the EGL origin
- SHH pathway inhibitors could be used for treatments.
- Then rest ~80% of human medulloblastoma ?

  (~15% WNT signaling/

  ~10% N-myc overexpression)

Cancer Research 63(17):5428

AJOU GIRC Genomic Instability Research Center
유전체불안정성 제어 연구센터

Summary

- ***Xrcc1*** **deficiency selectively targets interneuron populations in the cerebellum.**
- **Genomic instability resulting from Xrcc1 inactivation leads to apoptosis in the external germinal layer (EGL) while triggers cell cycle arrest in the white matter (WM) during postnatal cerebellum development.**
- **Genomic instability due to DNA damage repair defects results in medulloblastoma formation in a *p53* deficient background.**
- **Abnormal SHH signaling is a major driving force to induce medulloblastoma with genomic instability.**