

# NIR & Children's Health



Ljubljana, 18-20 may, 2011

Radiofrequencies  
Experimental Studies, Mechanisms of Interaction

Carmela Marino



ENEA, Unit of Radiation Biology and Human Health, Rome, Italy

*Cost action BM0704*

Working Group 4: EMF - Biology  
Carmela Marino, Isabelle Lagroye,  
Claudio Pioli, Maria Rosaria Scarfi and Zenon  
Sienkiewicz,

# Review of literature

About 40 peer reviewed research papers

## Cell types investigated

### Humans

- Umbilical cord blood-derived
- Fibroblasts from skin biopsies of a child
- Neuroblastoma cell lines
- Fibroblasts from fetus
- Trophoblast cell line
- Amniotic cells

### Rodents

- Primary neurons
- Primary astrocytes
- Neuroblastoma
- Embryonal-derived neural cells
- Embryonal fibroblasts
- Embryonal carcinoma
- Stem cells
- Chick embryos

## RF Radiation exposure

Frequency range: 835 – 2 450 MHz

SAR: from 80 mW/kg to 100 W/kg

Exposure duration: from 30 min to 10 weeks

Signals: CW, CDMA, FDMA, GSM basic, GSM-talk; GSM-DTX; DAMPS-835; IMT-200

Exposure working-mode: continuous, intermittent (on/off cycles)

## Biological targets examined

Apoptosis

Viability

Cell cycle

Proliferation

Differentiation

Cytosolic Ca<sup>2+</sup> spiking

Gene and protein expression

Genotoxicity, cytogenicity

DNA migration

Neoplastic transformation

## Results

- In most cases no effects of RF radiation exposure (<100 W/kg)
  - Mixed effects of co-exposures
- No genotoxic effects in 10/13 studies (77%)
- Transient genotoxic effects (recovery after short times) in 2/3 positive studies (67%)
- No neoplastic transformation
- Effects on gene expression not associated to variation in related protein expression or to detectable changes in cell physiology

## Gaps in knowledge

- channels, enzyme or specific pathways related to differentiation and proliferation involved in embryogenesis to help the understanding of the interaction mechanism studies.
- specific clones
- Modeling the response of cancer stem cells?
- proteomics analysis (gene expression) in stem cells (human stem cell line)

# Animal studies investigating effects on brain structure and function (1/7)

Reference	Model used	Exposure conditions	Results of exposure
Finnie et al, 2006a	<i>c-fos</i> expression in fetal BALB/c mouse brain	GSM 900 MHz; 1 h/day from day 1 to 19 of gestation at 4 W kg <sup>-1</sup>	No significant effect in pyriform cortex or basal ganglia
Finnie et al, 2009	HSP25, 32, 70 expression in fetal BALB/c mouse brain	GSM 900 MHz; 1 h/day from day 1 to 19 of gestation at 4 W kg <sup>-1</sup>	No effects (HSPs induced by hyperthermic shock)
Kumlin et al, 2007	PCNA, pCREB, in young Wistar rat brain	GSM 900 MHz; 2h/day, 5 weeks at 0.3 or 3 W kg <sup>-1</sup> ; animals freely moving	No significant effects on hippocampus or dentate gyrus

# Animal studies investigating effects on brain structure and function (2/7)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Odaci et al, 2008	Histology of the dentate gyrus in Wistar rat, 4 weeks of age	900 MHz, CW; 90 min/day; conception until birth, at 2 W kg <sup>-1</sup>	Significant decrease in total number of granule cells	Data from 3 litters per treatment
Bas et al, 2009b	CA1 area of hippocampus in Wistar rat, 4 weeks of age	900 MHz, CW; 90 min/day; coception until birth, at 2 W kg <sup>-1</sup>	Significant decrease in total number of pyramidal cells.	Data from 3 litters per treatment
Bas et al, 2009a	Pyramidal cell numbers in Wistar rat hippocampus, using optical fractionator technique	GSM 900 MHz; 1 h/day for 28 days at 0.016 W kg <sup>-1</sup> , 2 W kg <sup>-1</sup> in head	Significant decrease in pyramidal cell numbers; increase in dark neurons	Animals aged 12 weeks at start: considered developmentally equivalent to teenagers

# Animal studies investigating effects on brain structure and function (3/7)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Sonmez et al, 2010	Purkinje cells in Wistar rat cerebellum	900 MHz, CW; 1 h/day; 28 days at 0.016 W kg <sup>-1</sup> , 2 W kg <sup>-1</sup> in head	Significant decrease in number of Purkinje cells.	Also no effect on body or brain weights
Rağbetli et al, 2009, 2010	Cell numbers, Swiss albino mouse brain on postnatal day 21	GSM 900 MHz; at 1.2 or 0.95 W kg <sup>-1</sup> from mobile phone, in standby mode 11h 45 min and in talk mode 15 min per day, dg 1-20	Significant decrease in Purkinje cells in cerebellum, no effect on pyramidal cells in hippocampus	Dosimetric basis of reported SAR value unclear; only 5 or 6 animals per treatment group

# Animal studies investigating effects on blood-brain barrier (3/4)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Kuribayashi et al, 2005	Permeability to albumin using FITC-dextran, in 4 or 10 week old Fisher 344 rat, expression of claudin-5, auaporine-4, p-glycoprotein, by immunohistochemistry (IHC), q RT-PCR, pathological changes	PDC 1.439 GHz, 6.7 ms pulses at 50 pps; 90 min/day, 6 days/week for 1 or 2 weeks at 2 or 6 W kg <sup>-1</sup> in brain	No significant effects on permeability or gene expression	1,3-dinitrobenzene (10 mg/kg) increased leakage and decreased gene expression

# Animal studies investigating effects on blood-brain barrier (4/4)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Finnie et al 2006b, 2006c	Permeability to Evans blue in near-term, neonatal BALB/c mouse brain, using IHC	GSM 900 MHz; 60 min/day on gestational days 1-19 or on postnatal days 1-7 at 4 W kg <sup>-1</sup>	No significant effects on albumin leakage	Chemical control gave positive results
Kumlin et al, 2007	Permeability to Evans blue in immature Wistar rat brain	GSM 900 MHz; 2 h/day, 5 days/week for 5 weeks at 0.3 or 3 W kg <sup>-1</sup> ; animals freely moving	No significant effects on leakage	Examined in 35 µm sections

# Animal studies investigating effects on behaviour (1/3)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Kumlin et al, 2007	Performance of Wistar rats in Morris water maze; 4 trials/day for 4 days, probe trial 24 h later	GSM 900 MHz; 2 h/day from postnatal day 24 for 5 days/week for 5 weeks at 0.3 or 3 W kg <sup>-1</sup>	Improved performance: significantly decreased escape times; significantly increased time in platform quadrant during probe trial at 3 W kg <sup>-1</sup>	Also no effects on brain morphology, numbers of dark neurons or on BBB permeability

# Animal studies investigating effects on behaviour (2/3)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Takahashi et al, 2010	<p>Fertility, pregnancy outcome, abnormalities, malformations, growth, physical and reflex development, in Crl:CD(SD) rats.</p> <p>Behaviour of offspring in open field at 5, 8 weeks, spatial memory in a water maze at 9 weeks, fertility and embryofetal losses in pregnant animals at 10 weeks</p>	<p>W-CDMA down-link signals 2.14 GHz; 20/day from dg 7 to postnatal day 21, at 0.028-0.040 or 0.066-0.093 W kg<sup>-1</sup> in mothers, 0.029 or 0.068 W kg<sup>-1</sup> in fetus, 0.061-0.067 or 0.143-0.156 in offspring, animals freely moving</p>	<p>No significant effects on mothers or offspring, except time in target quadrant of water maze increased for males during probe trial</p>	<p>A few significant effects not considered to be of biological significance</p>

# Animal studies investigating effects on behaviour (3/3)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Gagnon et al, 2003	Histology of thymus, adrenal, haematology, corridor behaviour, in Swiss Webster mice, at 21 days old	0-25 MHz broadband signals; 24 h/day from dg 18 – postnatal day 21, at 6.8 V (abstract says 12.8 V) animals freely moving, in home cages	Increased numbers of animals with lesions; time to run corridor almost doubled	Paucity of field and exposure details. Limited statistical analysis. Changes in total white blood cell and absolute lymphocyte counts significantly elevated in both exposed and sham mothers

# Animal studies investigating effects on auditory function (1/3)

<b>Reference</b>	<b>Model used</b>	<b>Exposure conditions</b>	<b>Results of exposure</b>	<b>Comments</b>
Kizilay et al, 2003	DPOAE (1-6.3 kHz) in newborn and adult SD rats, measured before and after exposure	GSM 900 MHz; 1 h/day for 30 days at 0.95 W kg <sup>-1</sup>	No significant differences	Exposure started on day 2. Otomicroscopy revealed no pathologies
Kayabasoglu et al, 2010	DPOAE (1-8 kHz) in newborn and adult Wistar rats, before and after exposure	GSM 900 or 1800 MHz signal from mobile phones; 6h/day for 30 days; using carousel system	No significant effect compared to unexposed control animals	SAR not given, no dosimetry. Outputs of phones rated at 0.85-0.93 W kg <sup>-1</sup>

# Animal studies investigating effects on auditory function (2/3)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Budak et al, 2009a	DPOAE (1-8 kHz) in 1 month old and adult female NZW rabbits, measured in both ears after exposure	GSM 1800 MHz, shielded chamber, an horn antenna; 15 min/day for 7 days at 0.1 W	DPOAE amplitudes significantly increased in young (at 1, 1.5, 2, and 6 kHz) and significantly decreased in adults (at all frequencies)	SAR not given, no dosimetry
Budak et al, 2009b	DPOAE (1-8 kHz) in NZW rabbits, measured in both ears after exposure (age not specified – 6 weeks old?)	GSM 1800 MHz; 0.1W 15 min/day pre and or post-natal exposure	DPOAE significantly increased in pre (at 1.5 kHz), and in pre+post (at 1.5, 3 and 6 kHz), and significantly decreased in post (at 4 and 6 kHz).	SAR not given, no dosimetry on pregnant or young animals

# Animal studies investigating effects on auditory function (3/3)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Budak et al, 2009c	DPOAE (1-8 kHz) in pregnant and non-pregnant NZW rabbits, measured in both ears after exposure	GSM 1800 MHz; 15 min/day for 7 days (gestational days 15-22) at 0.1 W; animals anaesthetised	DPOAE significantly decreased in non-pregnant (at 1-4 kHz). No significant decrease in pregnant (except at 2 kHz)	SAR not given, no dosimetry. Large variability in results

# Animal studies investigating teratological effects (1/4)

<b>Reference</b>	<b>Model used</b>	<b>Exposure conditions</b>	<b>Results of exposure</b>	<b>Comments</b>
Lee et al, 2009	Pregnancy outcome, abnormalities, malformations, in ICR mice, on gestational day 18	CDMA 849 MHz, WCDMA 1.95 GHz; 2x45 min/day on gestational day (dg) 1 to 17, at 2 W kg <sup>-1</sup>	No significant effects on mothers or offspring with CDMA or both signals	Exposure separated by 15 min interval. All exposures not associated with temperature increase.
Ogawa et al, 2009	Pregnancy outcome, abnormalities, malformations, in CD(SD) rats, on gestational day 20	W-CDMA 1.9 GHz; 90 min/day on dg 7 to 17, at 0.67 or 2 W kg <sup>-1</sup> (in maternal brain)	No significant effects on mothers or offspring	Whole body SAR of fetus approx half that of mother (<0.4 W kg <sup>-1</sup> )

# Animal studies investigating teratological effects (2/4)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Sommer et al, 2009	Fertility, pregnancy outcome, abnormalities, malformations in C57BL mice, over 4 generations	UMTS 1.966 GHz; 23.5 h/day, SARs 0.08, 0.4 or 1.3 W kg <sup>-1</sup> ); animals freely moving	No significant effects except for trend towards lower food consumption in exposed males, few sporadic changes	SARs for 3 adult mice per exposure cage.
Contalbrigo et al, 2009	Glucose, triglycerides, cholesterol, in plasma of SD rats, every 3 h, using automatic analyser	GSM 1800 MHz; 19 h/day, from dg 12 until 56 weeks of age, at 25, 50 V m <sup>-1</sup>	No significant effects	No description of exposure system; no dosimetry. No measure of variability on data

# Animal studies investigating teratological effects (3/4)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Fragopoulou et al, 2010b	Skeletal anatomy in BALB/c mice, at birth and at 35 days	GSM 900 MHz signal mobile phone in talk mode; 6 or 30 min/day from dg 0 to day 21, at 0.6-0.94 W kg <sup>-1</sup>	Delay in ossification in cranial bones and thoracic ribs. No effect seen at 35 days	Females exposed to GSM signal for 6 or 30 min/day for 5 days immediately before pregnancy
Pyrpasopoulou et al, 2004	Histology, expression of bone morphogenetic proteins receptor subunits in Wistar rat kidney at birth	9.4 GHz, pulsed length of 20 s and pulse rate of 50 Hz; continuously on dg 1-3 or 4-7, at 0.0005 W kg <sup>-1</sup> (0.05 W m <sup>-2</sup> ) animals freely moving	Significant changes in expression of BMP-4, BMPR-IA, BMPR-II; effects more pronounced on days 1-3	Signal scaled to rat dimensions in order to produce equivalent penetration as a GSM signal in man. Paucity of exposure details

# Animal studies investigating teratological effects (4/4)

<b>Reference</b>	<b>Model used</b>	<b>Exposure conditions</b>	<b>Results of exposure</b>	<b>Comments</b>
Gul et al, 2009	Morphology of ovarian follicles in (unspecified) rats, using microscopy, image analysis, at postnatal day 21	Unspecified signal from mobile phone beneath cage; in stand-by mode for 11 h 45 min/day and talk mode for 15 min/day, from dg 0 to birth, animals freely moving	Significant decreases in mean number of pups per litter, and in ovarian number and volume	No dosimetry. Phone battery charged continuously

# Animal studies investigating effects on the immune system

Reference	Model used	Exposure conditions	Results of exposure	Comments
Sambucci et al, 2010	Pregnancy outcome, B cell functions, antibody production in C57BL/6 mice at 5 and 26 weeks of age	2.45 GHz, pulsed WiFi signal, 2 h/day, dg 5-19, 4 W kg <sup>-1</sup> , animals restrained	Please, wait until 10.30 and see the presentation from Claudio Pioli with results from these papers and more ...	
Laudisi et al. Manuscript in preparation	Effects of prenatal exposure on T cell differentiation and functions			

# Animal studies investigating effects on genotoxicity and mutagenicity

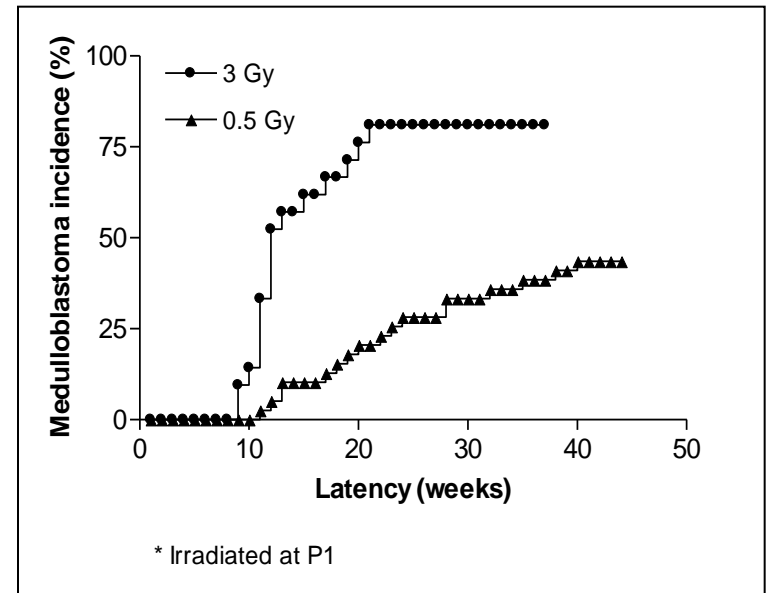
Reference	Model used	Exposure conditions	Results of exposure	Comments
Vijayalaxmi et al, 2003	Micronuclei (MN) in 2000 polychromatic erythrocytes (PCE) in bone marrow of Fischer 344 rats, up to 9 days after exposure	Iridium 1.6 GHz signals; for 2h/day, 7 days/week from gestational day 19 until postnatal day 35, at 0.1-0.22 W kg <sup>-1</sup> in brain; animals freely moving	No significant increase in incidence	Mitomycin C (MMC; 0.01 mg/kg) caused significant increase, 24 h after injection. Animals part of carcinogenicity study (Anderson et al, 2004)
Ferreira et al, 2006	MN in 1000 PCE in bone marrow cells of Wistar rats, on postnatal day 2	834 MHz; 8.5 h/day from gestational day 0 to birth; 26.8-40 V m <sup>-1</sup> from phone, SAR of 0.55-1.23 W kg <sup>-1</sup>	Significant increase in MN	Animals exposed from 17:30 to 02:00. Also no significant effects on catalase, glutathione or other antioxidant functions in liver or blood

# Animal studies investigating effects on cancer

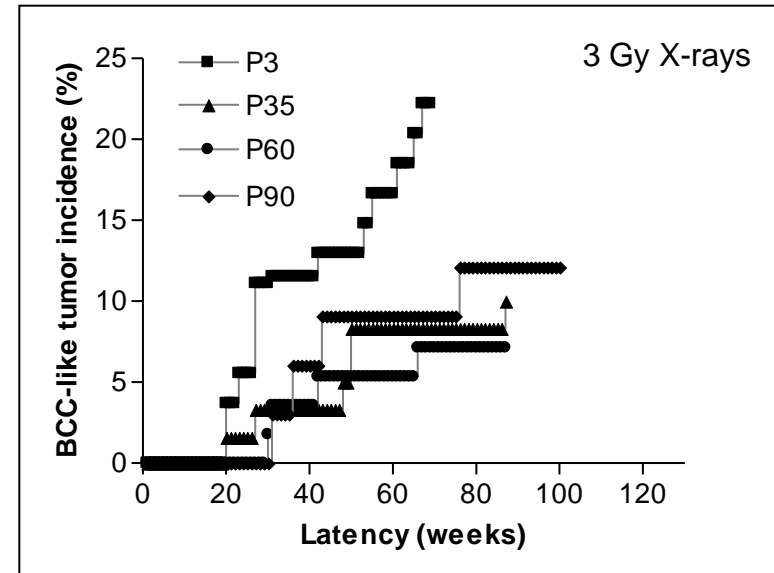
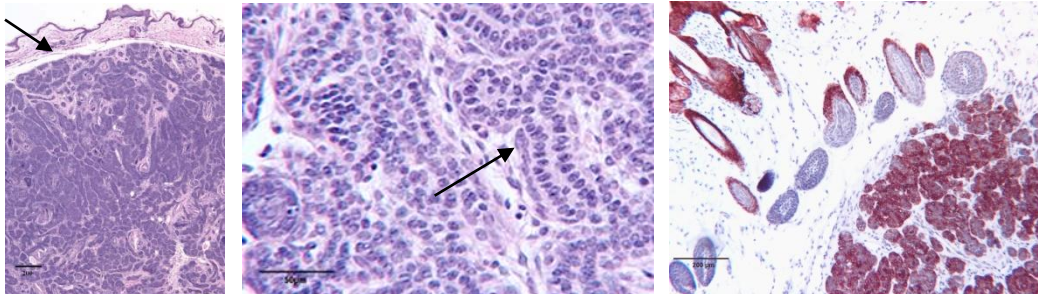
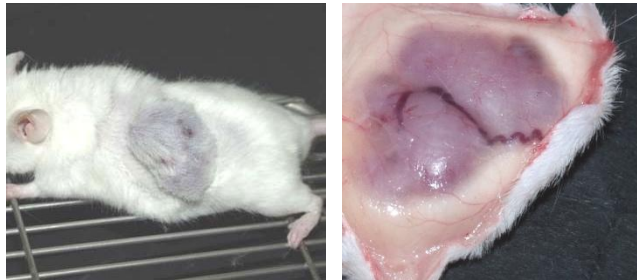
Reference	Model used	Exposure conditions	Results of exposure	Comments
Jin et al, 2010	Weight gain, survival, in SD rats, urinalysis, haematology, blood biochemistry after exposure, tumour incidence by <i>post mortem</i> pathology	CDMA 849 MHz, WCDMA 1.95 GHz; 45 min/day, 5 days/week for 1 year, at 2 W kg <sup>-1</sup> (per signal) animals freely moving	No cancer-related effects; Changes in some haematological analyses	Exposures am or pm alternately.
Saran et al, 2007	Multiple tumours (medulloblastomas, rhabdomyosarcomas and preneoplastic lesions typical of basal cell carcinomas) in <i>Patched1 (Pct1)</i> heterozygous mice, by <i>post mortem</i> pathology	GSM 900 MHz; 2X30 min/day for 5 days, from postnatal day 2 to 6, at 0.4 W kg <sup>-1</sup> ; animals restrained in polystyrene jigs	No significant decrease in survival; no significant increase in incidence, onset or histology of tumours, or in preneoplastic lesions.	<i>Pct1</i> mice show peak sensitivity to X-rays during early postnatal life.

# X-rays *Ptch1*-dependent tumors

## Medulloblastoma



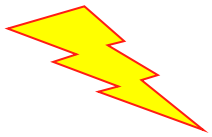
## Basal Cell Carcinoma



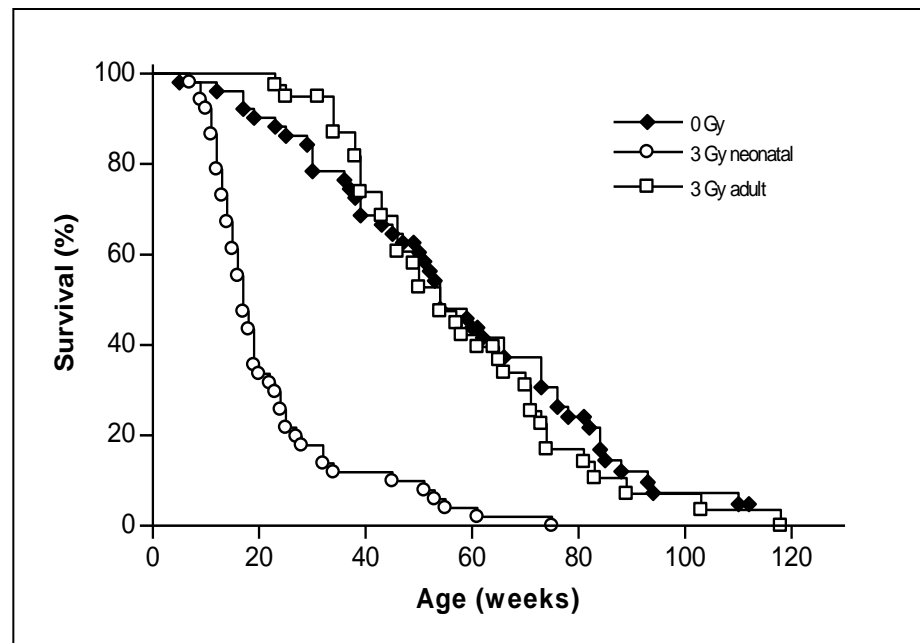
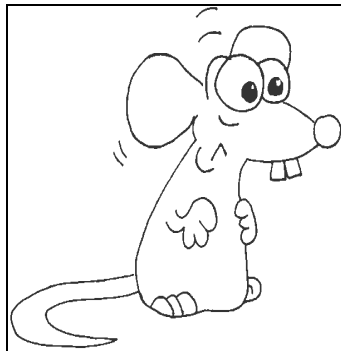
# Ionizing radiation hypersensitivity

X-rays irradiation of neonatal *Ptch1* heterozygotes dramatically increases the incidence of medulloblastoma (81%) over the spontaneous rate (7%) and induce basal cell carcinoma development. Thus, newborn *Ptch1* heterozygous mice constitute an extremely sensitive mouse model of radiation-induced tumorigenesis and represent a useful tool to evaluate the detrimental effects of exposure to potentially harmful agents.

250 kVp X Rays

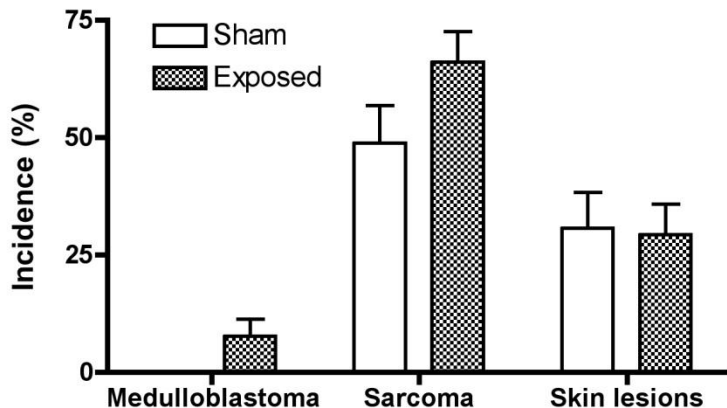
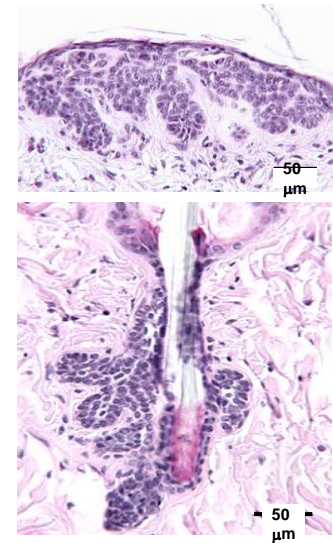


3 Gy



## Tumorigenesis in *Ptch1*<sup>+/-</sup> mice exposed to RF

	Number of autopsied mice	Observed lesions (% ± SE)			
		Medulloblastoma	Sarcoma	Basal cell carcinoma	Preneoplastic skin lesions
<b>Sham</b>					
Male	19	-	8 (42.1 ± 11.3)	-	5 (29.4 ± 11.1)
Female	20	-	11 (55.0 ± 11.1)	-	6 (31.6 ± 10.7)
<b>Total</b>	<b>39</b>	<b>-</b>	<b>19 (48.7 ± 8.0)</b>	<b>-</b>	<b>11 (30.6 ± 7.7)</b>
<b>Exposed</b>					
Male	22	2 (9.1 ± 6.1)	12 (54.6 ± 10.6)	-	5 (25.0 ± 9.7)
Female	31	2 (6.5 ± 4.4)	23 (74.2 ± 7.9)	-	9 (32.1 ± 8.8)
<b>Total</b>	<b>53</b>	<b>4 (7.6 ± 3.6)</b>	<b>35 (66.0 ± 6.5)</b>	<b>-</b>	<b>14 (29.2 ± 6.6)</b>



No significant statistical differences in *Ptch1*-dependent tumorigenesis were observed between sham and exposed mice.

# Animal studies investigating teratological effects (4/4)

Reference	Model used	Exposure conditions	Results of exposure	Comments
Tillmann et al., 2010	female B6C3F1	UMTS fields with intensities of 0, 4.8, and 48 W/m(2), the low-dose group (4.8 W/m(2)) was subjected to additional prenatal ethylnitrosourea treatment (40 mg ENU/kg body weight).	cocarcinogenic effect of lifelong UMTS exposure (4.8 W/m(2)) in descendants subjected to pretreatment with ethylnitrosourea	tumour multiplicity of the lung carcinomas was increased and the number of metastasising lung tumours was doubled in the ENU/UMTS group as compared to the ENU control group

## **Gaps in knowledge**

- other end-points (hormonal level for different glands)
- Appropriate mouse models related with children and childhood disease: glioblastoma, medulloblastoma, retinoblastoma....prone mice/rats (check the existing models or generate new strains)
- Animal two-hit models
- Mechanistic predictors (tumor promotes)
- In silico tumor formation (modeling)

## **Gaps in knowledge**

- Paucity of published information on human cells
- Large variation in biological models, RF exposure and quality
- Lack of repetition studies
- Evaluation of co-exposures effects

THANK YOU