La Radioterapia guidata dall'Ossigeno

Francesco Pasqualetti

Associazione Italiana Radioterapia e Oncologia clinica
Società Italiana di Radiobiologia

DICHIARAZIONE

Relatore: Francesco Pasqualetti

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Consulenza ad aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Partecipazione ad Advisory Board **NIENTE DA DICHIARARE**
- Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **NIENTE DA DICHIARARE**

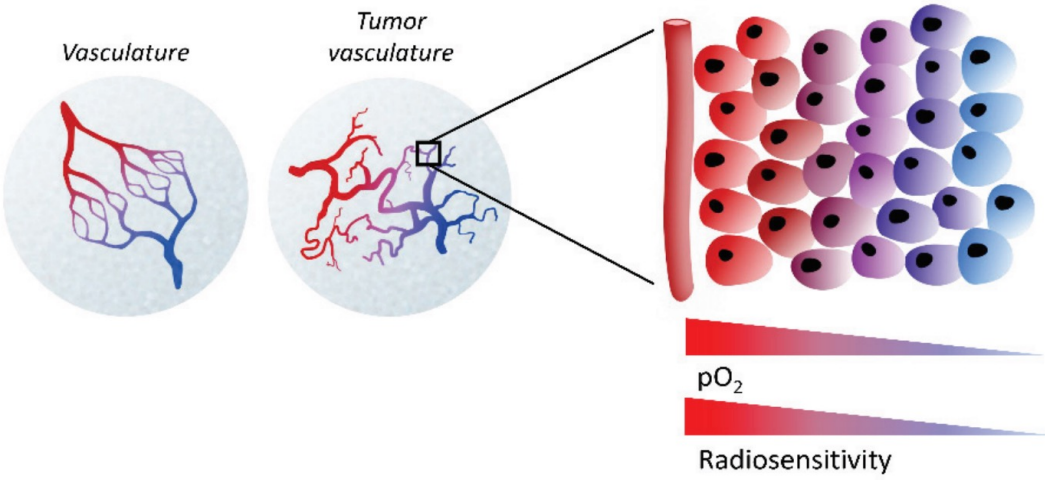
Hypoxia → is a condition in which the body or a region of the body is deprived of adequate oxygen supply at the tissue level.

The oxygen tension in normal tissues ranged from **30 to 52mmHg** (corresponds to 3.9 to 6.8% oxygen concentration in the gas phase at sea level)

Tumoral oxygen tension ranged in between **5.3 to 14mmHg** (0.7 to 1.8%), often with a considerable fraction of cells reaching below **5mmHg** (0.7%)

The majority of solid tumors are characterized by abnormal tumor vasculature.

When the high rate of tumor growth cannot be sustained by tumor angiogenesis, this causes limited oxygen supply to the tumor cells distal to the blood vessels, forming regions of hypoxia.



Genes 2015, 6, 935-956

Acta Oncol Stockh Swed. 2010;49(7):934–40

1950s → **Gray and Thomlinson** demonstrated by mathematical calculations, based on histological sections of human tumors, that regions around necrotic areas display a hypoxic oxygen gradient

Both hypoxia and necrosis were independently proven to be predictors of poor clinical outcome, independent of the tumor stage, histological grade and lymph node status

The first indications that a tissue poorly supplied with oxygen might be more resistant to ionizing radiation (IR) came about as early as in the **1920s**

1953 → Gray demonstrated on the preclinical level that a decrease in hypoxia, achieved by improving oxygen delivery, results in increased radiosensitivity.

Adv Exp Med Biol. 2009;645:241–6

Br J Cancer. 1955; 9(4):539–49

Proc R Soc Lond Ser B Contain Pap Biol Character. 1933;113(782):238–50.

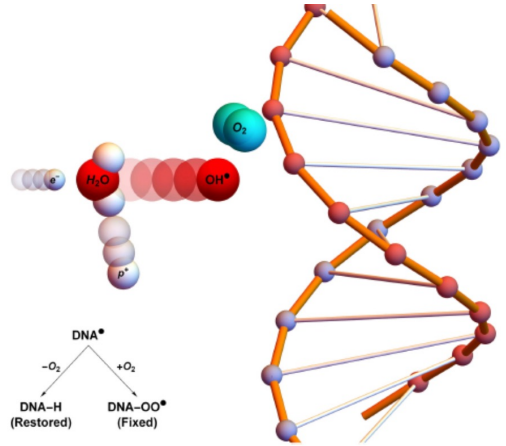
Oxygen fixation hypothesis

Photons ionize the atoms of the absorbing material and generate **free electrons**

These electrons mediate the IR-induced damage

- by directly damaging the macromolecules,
- by interacting with water in a process that producess hydroxyl (**OH•**) and hydrogen (**H•**) radicals

These short-lived and highly reactive molecules react with and damage the macromolecules

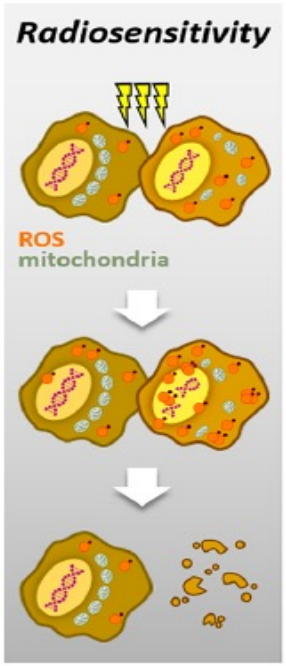


In absence of oxygen….
the extent of these reactions is limited due to the radicals'instability, and the majority of the damage is readily repaired

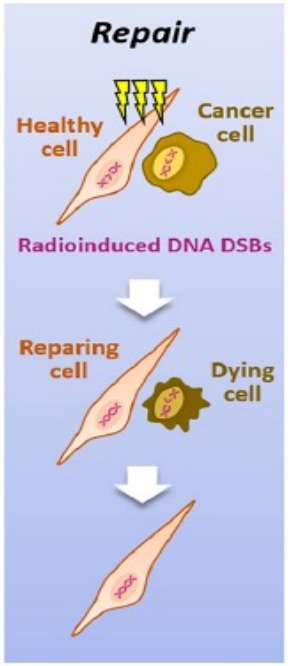
In presence of oxygen…

additional reactive oxygen species (ROS) are formed, e.g. hydrogen peroxides, which increase the overall concentration of DNA damaging agents.

Dr David Robert Grimes, University of Oxford, 4 Dec 2015



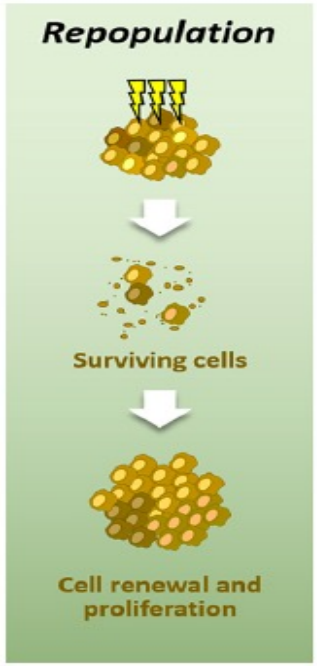
Oxygen effect
Stop OXPHOS
Stop Cell death
Autophagy



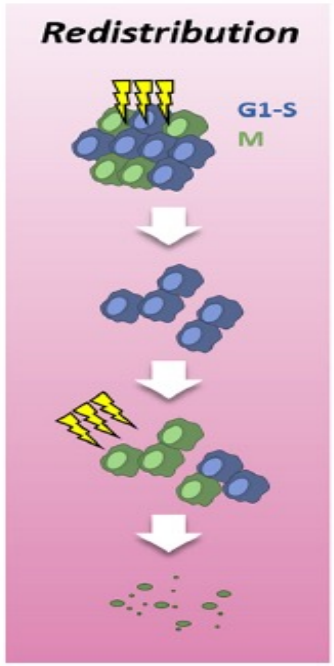
Down regulation Homologous recombination repair

Epigenetic modulations of DNA Damage Response

Up-regulation double-strand breaks

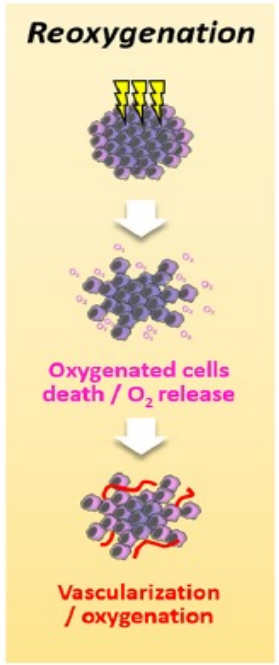


Improve cancer stem cells self-reneuwal

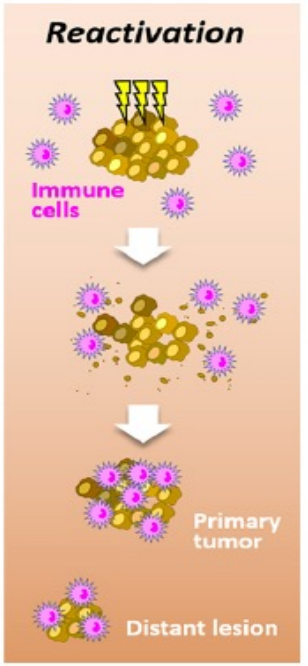


CSCs harbor a quiescent phenotype

More clles in G1 and s-phases

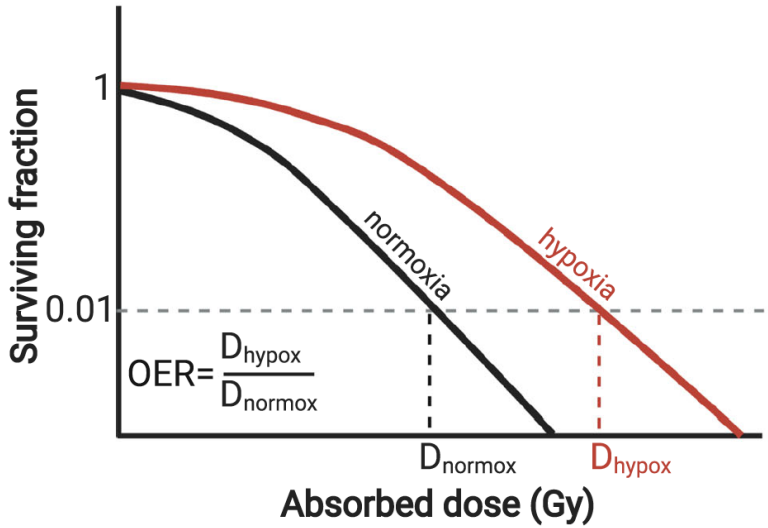


Oxidative stress that stabilizes HIF-1



Lactate accumulation leads to TAMs polarization toward an immuno-suppressive phenotype

Oxygen enhancement ratio (**OER**) → ratio between the **radiation dose under hypoxic conditions** and the **radiation dose under normoxic conditions** necessary to achieve the same amount of cell killing (determined in vitro by clonogenic assays)



Journal of Experimental & Clinical Cancer Research (2021) 40:197

Intratumoral pO_2 predicts survival in advanced cancer of the uterine cervix

Michael Höckel, Claudia Knoop, Karlheinz Schlenger, Birgit Vorndran, Evmarie Baußmann, Margarete Mitze, Paul Georg Knapstein and Peter Vaupel[a]

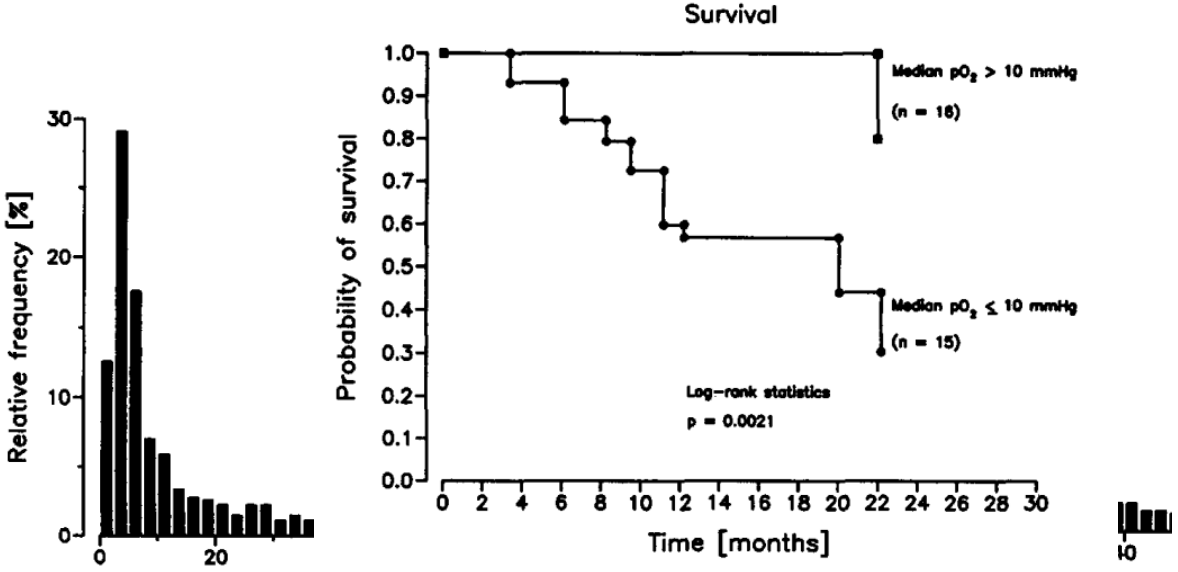


Fig. 2. Survival probability for patients with advanced cancer of the uterine cervix influenced by tumor oxygenation.

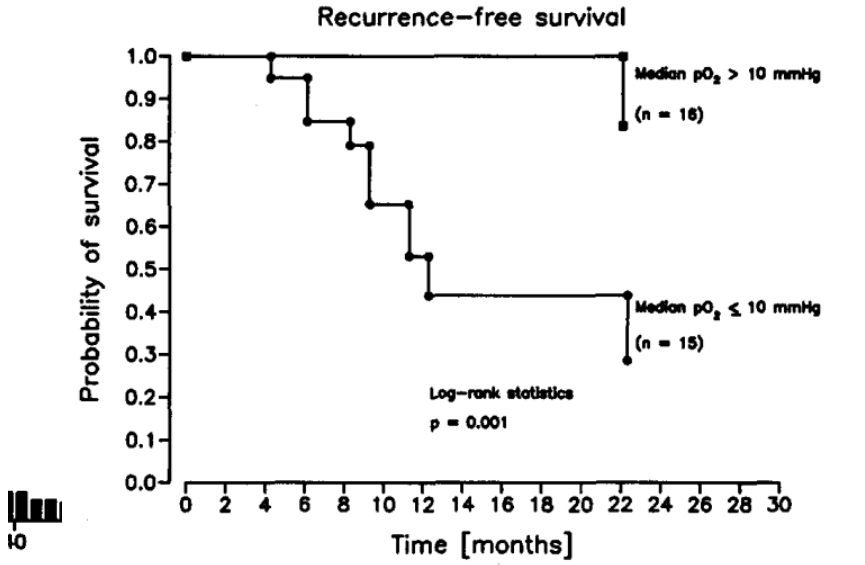


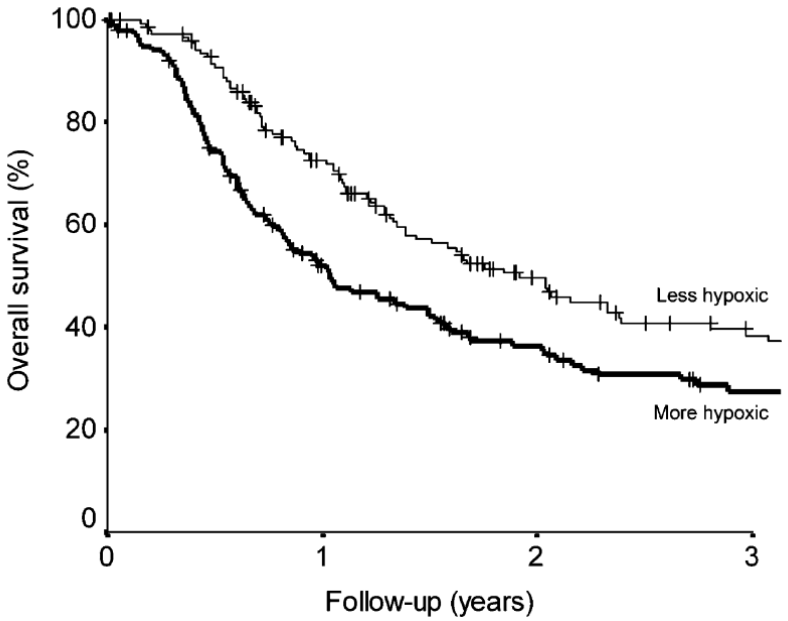
Fig. 3. Recurrence-free survival probability for patients with ad-

Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study

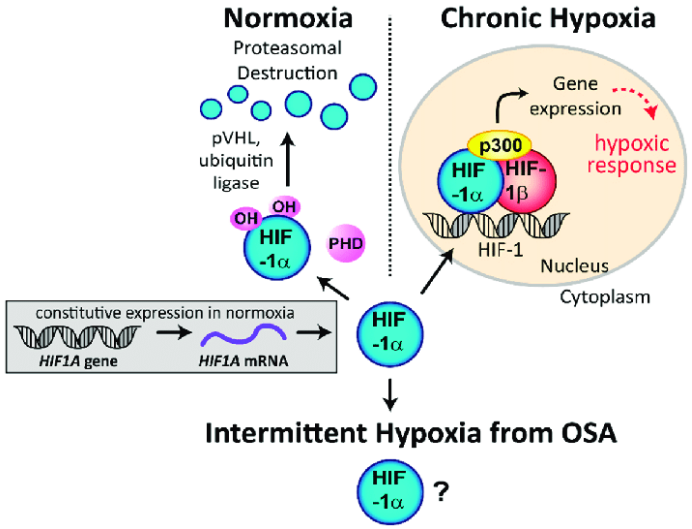
Marianne Nordsmark[a,*], Søren M. Bentzen[b], Volker Rudat[c], David Brizel[i], Eric Lartigau[d,e], Peter Stadler[f], Axel Becker[g], Markus Adam[h], Michael Molls[f], Juergen Dunst[g], David J. Terris[h], Jens Overgaard[a]

Tumor oxygenation, treatment characteristics and survival in 397 primary head and neck cancers

Center	N	Tumor oxygenation		
		Median pO_2 (mmHg) Median (range)	HP_5 (%) Median (range)	$HP_{2.5}$ (%) Median (range)
Aarhus	67	13 (0-54)	32 (0-100)	22 (0-95)
Paris	40	9 (0-55)	41 (0-100)	21 (0-97)
Halle	64	11 (0-59)	22 (0-89)	4.8 (0-73)
Heidelberg	54	9 (0-62)	41 (0-89)	22 (0-86)
Munich	62	9 (0-47)	41 (0-96)	25 (0-95)
Stanford	24	18 (0-51)	2 (0-94)*	0 (0-81)
Duke	86	5 (0-60)	51 (0-95)	-
All	397	9 (0-62)	38 (0-100)	19 (0-97)



Radiotherapy and Oncology 77 (2005) 18-24



HIF-regulated gene responses play key roles in various aspects of cancer development

Proliferation (**MYC**)
Angiogenesis (**VEGF, PDGF**)
Apoptosis/autophagy (**NDRG2, BNIP3**)
Metabolism (**PDK1, LDHA**)
DNA damage response (**GADD45A**)
Extracellular matrix remodeling (**LOX, MMP1**)
Cell migration and invasion (**CXCR4, SDF1**)

MiRNAs regulate gene expression

- A single miRNA → can target hundreds of different mRNAs
- Multiple cellular processes are controlled by miRNAs: proliferation, migration, invasion, apoptosis, differentiation, and drug resistance
- Hypoxia can modulate expression of several hypoxia-regulated microRNAs (HRMs), some of which, such as **miR-210, miR-26, and miR-181**, are directly controlled by hypoxia-inducible factor (HIF)

International Journal of Molecular Sciences 20(2):445
Huang et al., 2009; reviewed in Bertout et al., 2008; reviewed in Kaelin, 2008

updates

Hypoxia tolerance in the Norrin-deficient retina and the chronically hypoxic brain studied at single-cell resolution

Jacob S. Heng[a,b], Amir Rattner[a], Genevieve L. Stein-O'Brien[b,c], Briana L. Winer[b,c], Bryan W. Jones[d], Hilary J. Vernon[c], Loyal A. Goff[b,c], and Jeremy Nathans[a,b,e,f,1]

[a]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [b]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [c]McKusick–Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [d]Moran Eye Center, University of Utah, Salt Lake City, UT 84132; [e]Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and [f]Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205

CANCER GENOMICS

Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma

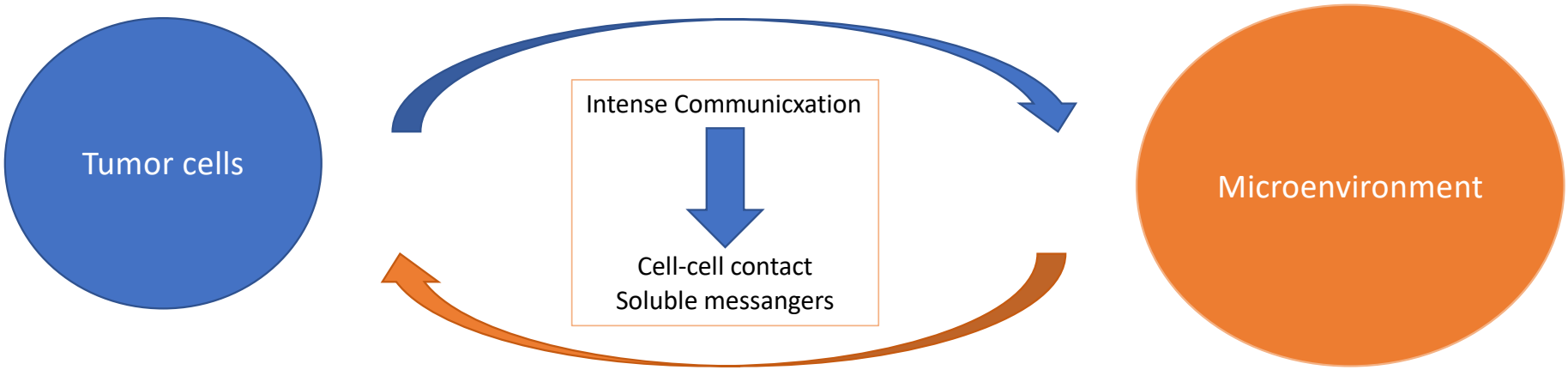
Anoop P. Patel,*[1,2,3,4] Itay Tirosh,*[3] John J. Trombetta,[3] Alex K. Shalek,[3] Shawn M. Gillespie,[2,3,4] Hiroaki Wakimoto,[1] Daniel P. Cahill,[1] Brian V. Nahed,[1] William T. Curry,[1] Robert L. Martuza,[1] David N. Louis,[2] Orit Rozenblatt-Rosen,[3] Mario L. Suvà,[2,3]†‡ Aviv Regev,[3,4,5]†‡ Bradley E. Bernstein[2,3,4]†‡

Check for updates

Hypoxia tolerance in the Norrin-deficient retina and the chronically hypoxic brain studied at single-cell resolution

Jacob S. Heng[a,b], Amir Rattner[a], Genevieve L. Stein-O'Brien[b,c], Briana L. Winer[b,c], Bryan W. Jones[d], Hilary J. Vernon[c], Loyal A. Goff[b,c], and Jeremy Nathans[a,b,e,f,1]

[a]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [b]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [c]McKusick–Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [d]Moran Eye Center, University of Utah, Salt Lake City, UT 84132; [e]Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and [f]Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205



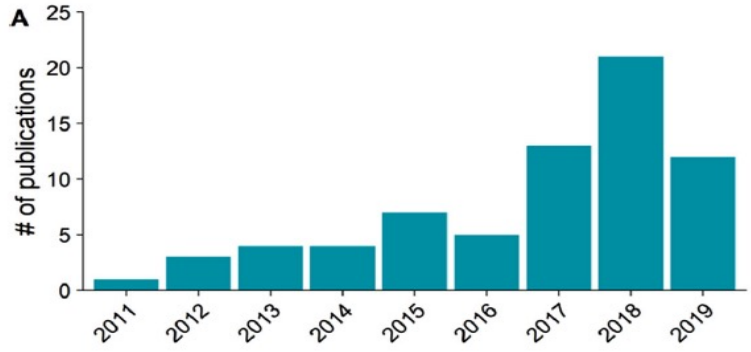
A variety of cytokines produced in the tumor microenvironment are released directly by **tumor cells** or by tumor associated cells (e.g., immune and stromal cells) and can regulate the expression of miRNAs involved in tumor pathogenesis and progression

Circulating miRNAs can be actively secreted outside the cell either encapsulated within **exosomes**

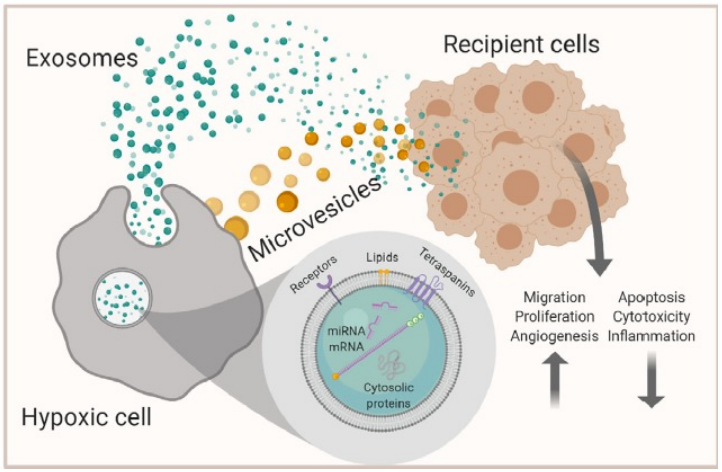
Aberrant levels of miRNAs can be detected not only in tumor cells but also in the biological fluids of cancer patients, **possibly reflecting the expression patterns of the tumor tissues from which circulating miRNAs originate**

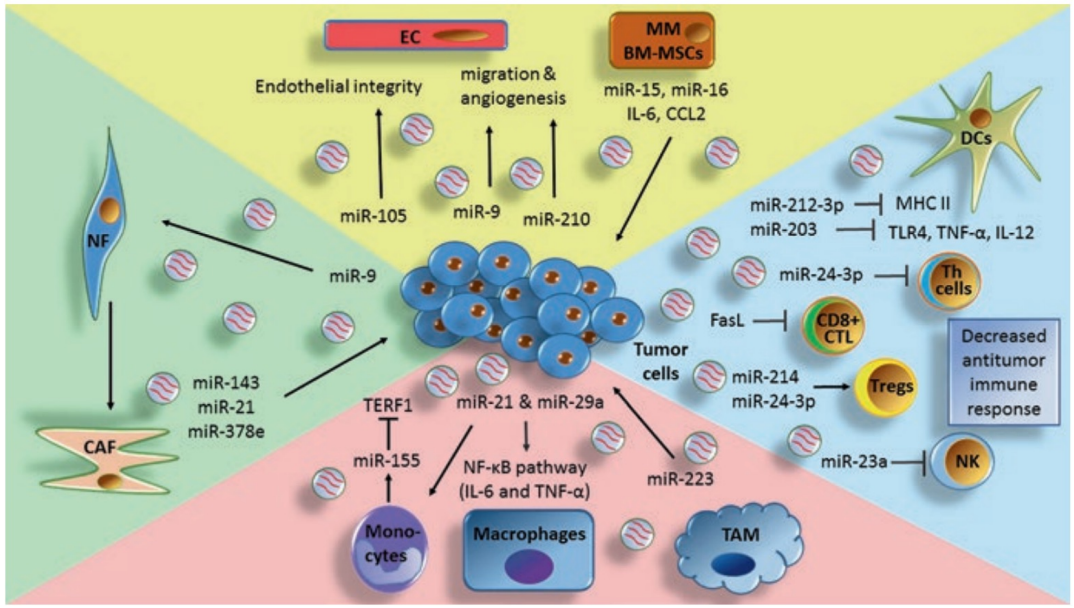
Journal of Hematology & Oncology, 2020, 13(1)
Nat Rev Cancer 4:11–22
Adv Exp Med Biol. 2018;1056:87-108

Exosome-mediated cell-to-cell communication in the tumor microenvironment



Number of publications on hypoxia induced extracellular vesicles (EVs)

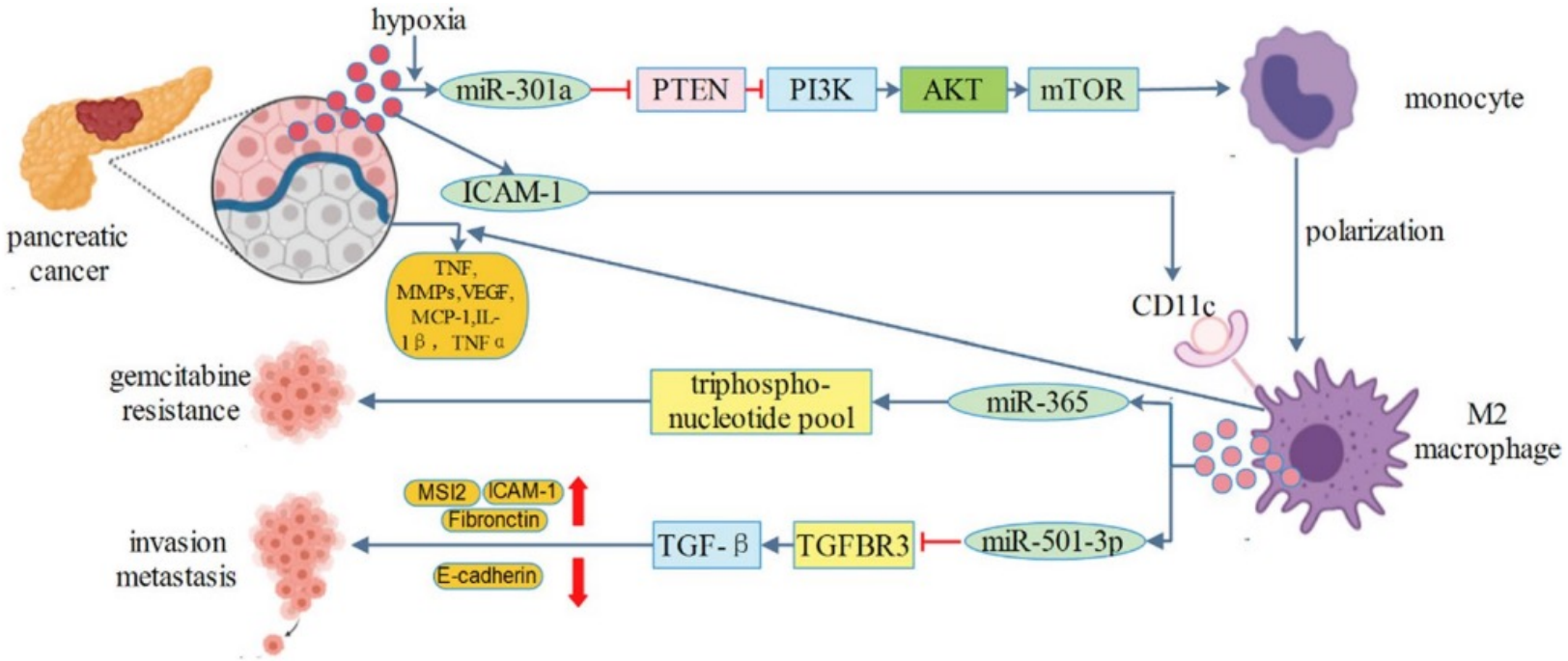




Journal of Hematology & Oncology, 2020, 13(1)
Nat Rev Cancer 4:11–22
Adv Exp Med Biol. 2018;1056:87-108

REVIEW Open Access

Exosomal microRNAs shuttling between tumor cells and macrophages: cellular interactions and novel therapeutic strategies



Journal of Cancer 2020, Vol. 11 4724

IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2020; 11(16): 4724- 4735. doi: 10.7150/jca.33232

Research Paper

Exosomes Derived from Hypoxic Colorectal Cancer Cells Transfer miR-410-3p to Regulate Tumor Progression

Xiufeng Hu*, Yu Mu*, Jie Liu, Xiaoqian Mu, Fangfang Gao, Lijuan Chen, Huijuan Wu, Hongbo Wu, Wenjing Liu, Yanqiu Zhao✉

Xu et al. *Cell Death and Disease* (2021)12:373
https://doi.org/10.1038/s41419-021-03664-1

Cell Death & Disease

ARTICLE Open Access

Hypoxic glioma-derived exosomes promote M2-like macrophage polarization by enhancing autophagy induction

Jianye Xu[1,2], Jian Zhang[1,2,3], Zongpu Zhang[1,2], Zijie Gao[1,2], Yanhua Qi[1,2], Wei Qiu[1,2], Ziwen Pan[1,2], Qindong Guo[1,2], Boyan Li[1,2], Shulin Zhao[1,2], Xiaofan Guo[1,2], Mingyu Qian[1,2], Zihang Chen[1,2], Shaobo Wang[1,2], Xiao Gao[1,2], Shouji Zhang[1,2], Huizhi Wang[1,2], Xing Guo[1,2], Ping Zhang[1,2], Rongrong Zhao[1,2], Hao Xue[1,2] and Gang Li[1,2]

Laboratory Investigation (2021) 101:612–624
https://doi.org/10.1038/s41374-020-00522-0

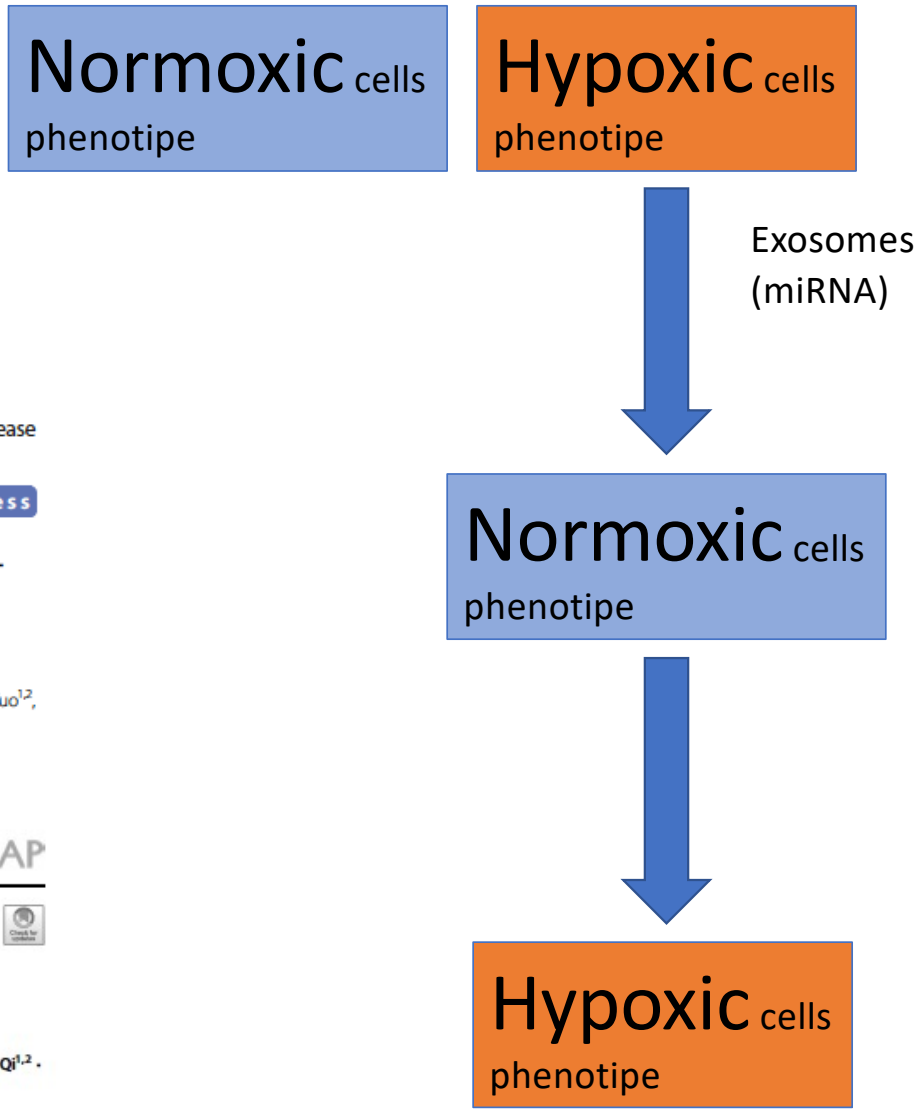
USCAP

ARTICLE

Exosomes derived from hypoxic glioma deliver miR-1246 and miR-10b-5p to normoxic glioma cells to promote migration and invasion

Mingyu Qian[1,2] · Zihang Chen[1,2] · Xiaofan Guo[1,2] · Shaobo Wang[1,2] · Zongpu Zhang[1,2] · Wei Qiu[1,2] · Yanhua Qi[1,2] · Shouji Zhang[1,2] · Jianye Xu[1,2] · Rongrong Zhao[1,2] · Hao Xue[1,2] · Gang Li[1,2]

Received: 26 December 2019 / Revised: 30 November 2020 / Accepted: 30 November 2020 / Published online: 14 January 2021



J Extracell Vesicles. 2020;10:e12002.

Hypoxic Cancer

Proliferation (**MYC**)
Angiogenesis (**VEGF, PDGF**)
Apoptosis/autophagy (**NDRG2, BNIP3**)
Metabolism (**PDK1, LDHA**)
DNA damage response (**GADD45A**)
Extracellular matrix remodeling (**LOX, MMP1**)
Cell migration and invasion (**CXCR4, SDF1**)

Bad

Good

Other Hypoxic scenarios

cerebrovascular disease
cardiovascular diseases
embryonic development
.....

Review

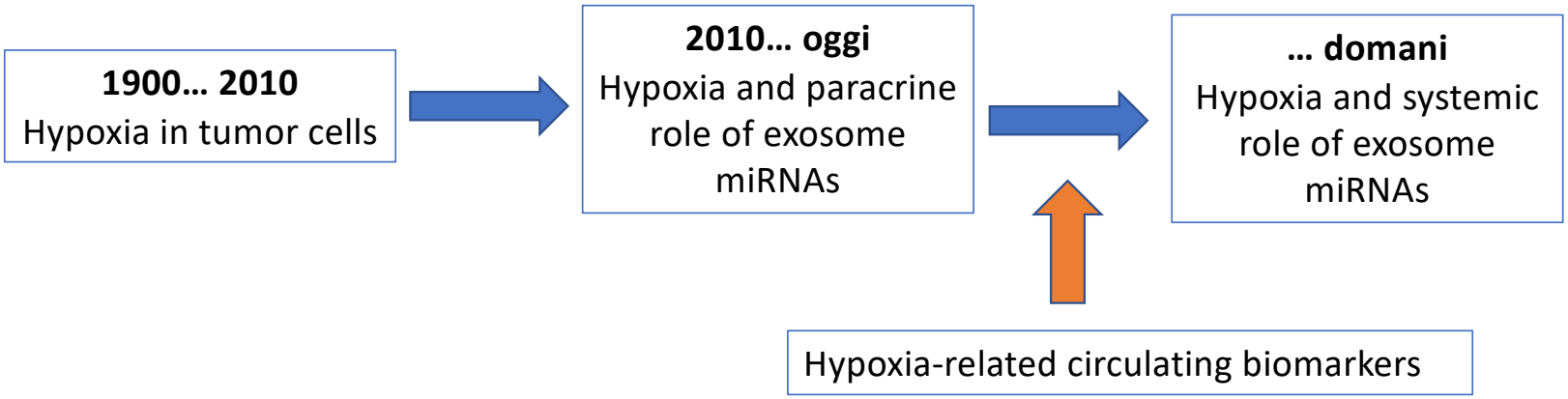
Circulating exosomes in cardiovascular disease: Novel carriers of biological information

Qing Liu [a], Hulin Piao [b], Yong Wang [b], Dongdong Zheng [b], Weitie Wang [b,*]

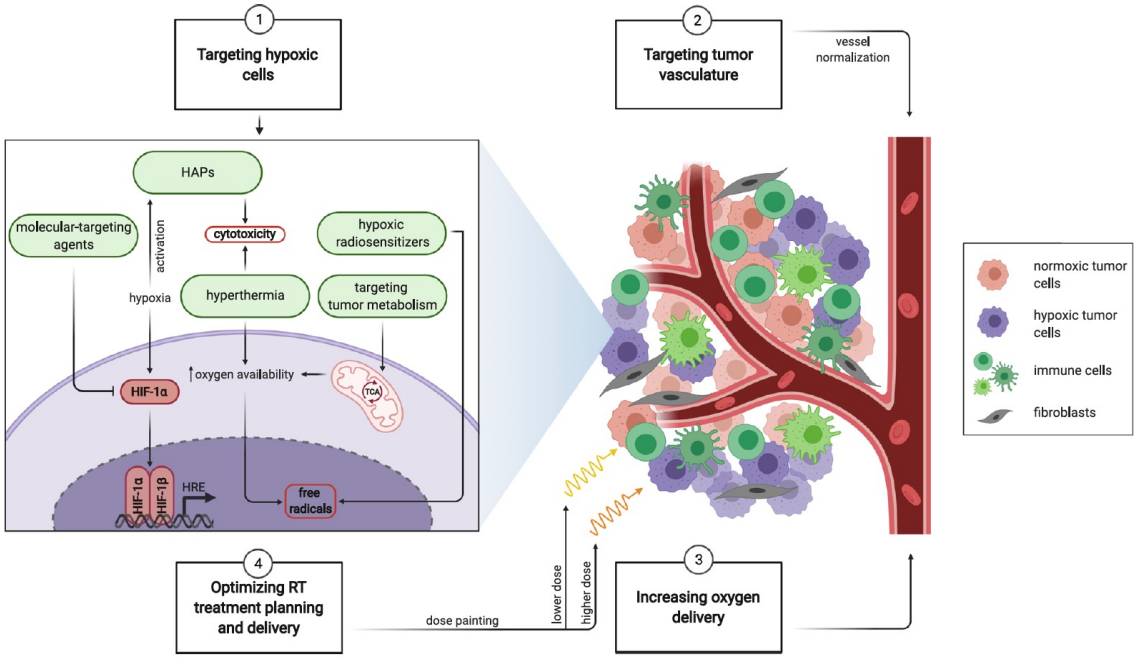
Exosomes and their content in cardiovascular disease.

Cardiovascular disease	Cargo in exosomes
Cardiac Fibrosis	WNT5a, TNF-α, let7-c, miR-19a, miR-21, miR-22, miR-132, miR-144, miR-146a, miR-181b, miR-210, miR-221, miR-294, LncRNA NONMMUT022555, lncRNA SRA1, LncRNA Mhrt, circ 000,203, circActa2
Ischemic Heart Disease	KLF5, miR-9−5p, miR-20b, miR-92a-3p, miR-128, miR-146a, miR-155, miR-155−5p, miR-221, miR-222, miR-223, miR301a-5p, miR-3129−5p, miR-320, miR-320d, miR-339, lncRNA GAS5
Myocardial Ischemia-Reperfusion Injury	HSP70, miR-21, miR-126,
Heart failure	miR-34a, miR-192, miR-194, miR-92b, miR-146a
Myocardial Infarction	miR-133a, miR-146a, miR-155, miRNA-214
Cardiac Hypertrophy	HSP20, miR-21, miR-200a

Hypoxia and Cancer



Targeting Tumor Hypoxia



Targeting Tumor Metabolism

Metformin
atovaquone
Papaverine

- Between the different targeting strategies, increasing oxygen availability by targeting oxidative phosphorylation is emerging as a particularly interesting approach
- Metformin has shown promising results in NSCLC and colorectal cancer patients receiving chemoradiotherapy (NCT02109549)
- There are numerous examples of oxidative phosphorylation inhibitors that have shown impressive results in cell lines and mouse models, but have not yet been able to advance to the clinical level.

Journal of Experimental & Clinical Cancer Research (2021) 40:197

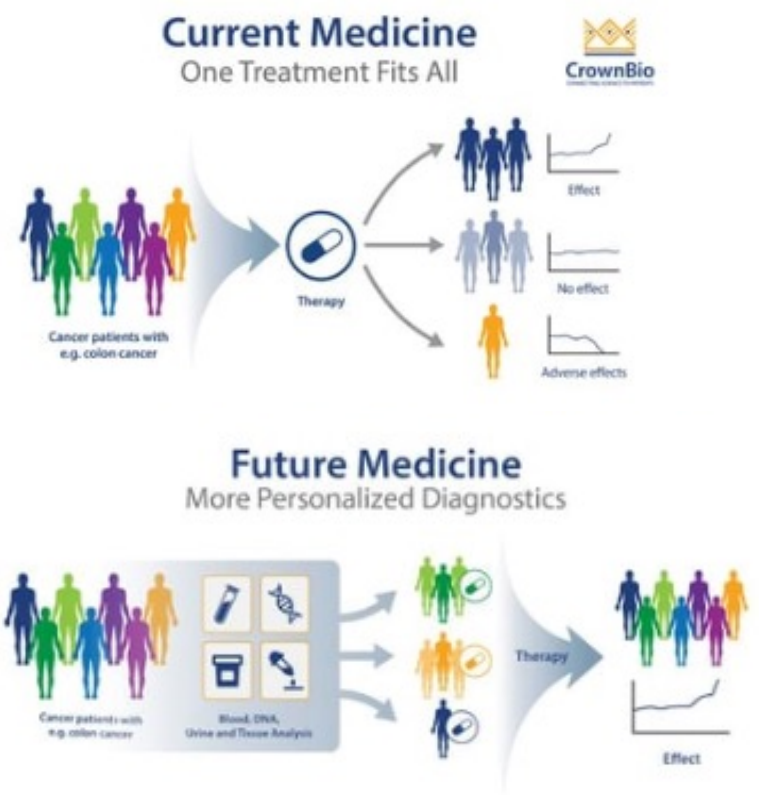
Personalized medicine → It is a medica model that separates people into different groups—with medical decisions, practices, interventions and/or products being tailored to the individual patient based on their predicted response or risk of disease.

Clin Cancer Res. 2021 May 01; 27(9): 2459–2469.

Mitochondrial Inhibitor Atovaquone Increases Tumor Oxygenation and Inhibits Hypoxic Gene Expression in Patients with Non-Small Cell Lung Cancer

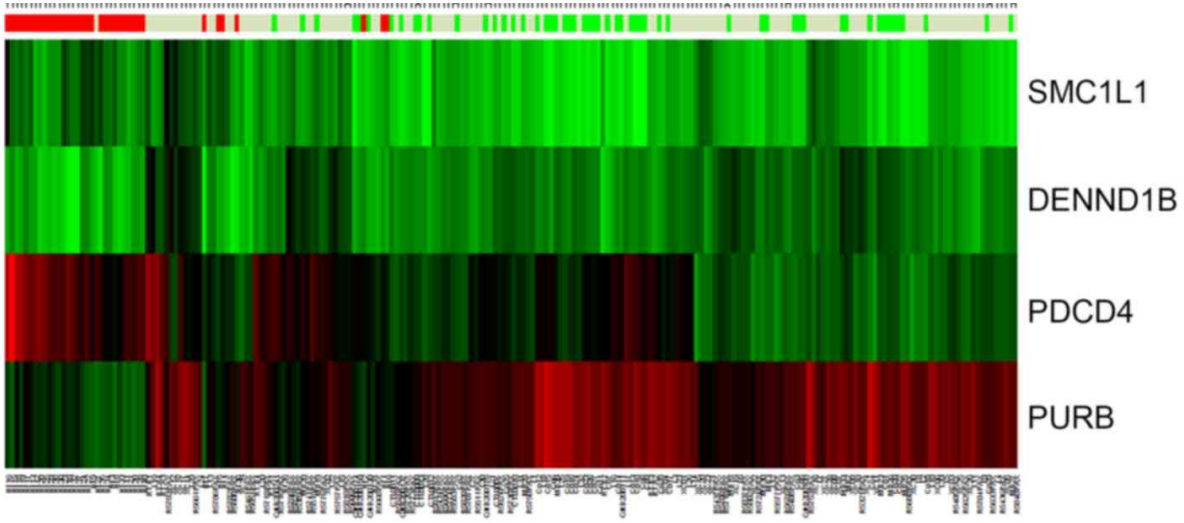
Michael Skwarski[1,2,*], Daniel R McGowan[1,3,*], Elizabeth Belcher[4], Francesco Di Chiara[4], Dionisios Stavroulias[4], Mark McCole[5], Jennifer L Derham[2], Kwun-Ye Chu[1,2], Eugene Teoh[2], Jagat Chauhan[6], Dawn O'Reilly[1], Benjamin HL Harris[1], Philip S Macklin[7], Joshua A Bull[8], Marcus Green[1], Gonzalo Rodriguez-Berriguete[1], Remko Prevo[1], Lisa K Folkes[1], Leticia Campo[1], Petra Ferencz[9], Paula L Croal[9], Helen Flight[10], Cathy Qi[11], Jane Holmes[11], James PB O'Connor[12], Fergus V Gleeson[13], W Gillies McKenna[1], Adrian L Harris[1], Daniel Bulte[9], Francesca M Buffa[1], Ruth E Macpherson[13], Geoff S Higgins[1,2]

Eligible patients were aged ≥18 years, had a pathological or radiological diagnosis of NSCLC, were scheduled for surgical resection, had disease >2 cm in diameter and were Eastern Cooperative Oncology Group (ECOG) performance status 0-2. Patients were excluded if taking known ETC inhibitors. Complete eligibility criteria are provided in the trial protocol (Supplementary Materials and Methods). All patients provided written informed consent.



Barbeau, J., (2018) *PDX and Personalized Medicine - Crown Bioscience*

Gene expression profiling is the measurement of the activity (the expression) of thousands of genes at once, to create a global picture of cellular function.



Tumor Hypoxia

Therapeutics, Targets, and Chemical Biology

Cancer Research

Development of a Hypoxia Gene Expression Classifier with Predictive Impact for Hypoxic Modification of Radiotherapy in Head and Neck Cancer

Kasper Toustrup[1], Brita Singers Sørensen[1], Marianne Nordsmark[1], Morten Busk[1], Carsten Wiuf[2], Jan Alsner[1], and Jens Overgaard[1]

They then evaluated a training set of 58 hypoxia-evaluated HNSCCs to generate a gene expression classifier containing **15 genes**.

This 15-gene hypoxia classifier was validated in 323 patients with HNSCC randomized for hypoxic modification or placebo in combination with radiotherapy.

Tumors categorized as hypoxic on the basis of the classifier were associated with a significantly poorer clinical outcome than nonhypoxic tumors.

This outcome was improved and equalized to the nonhypoxic tumors by addition of hypoxic modification (nimorazole).

Cancer Res; 71(17); 5923–31

Therapeutics, Targets, and Chemical Biology

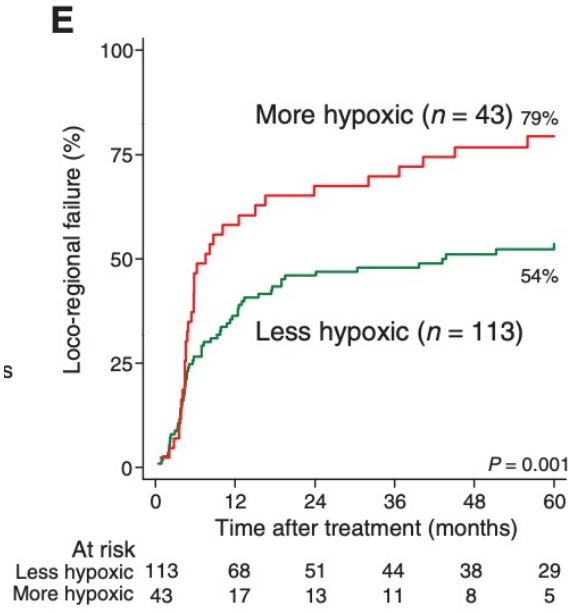
Cancer Research

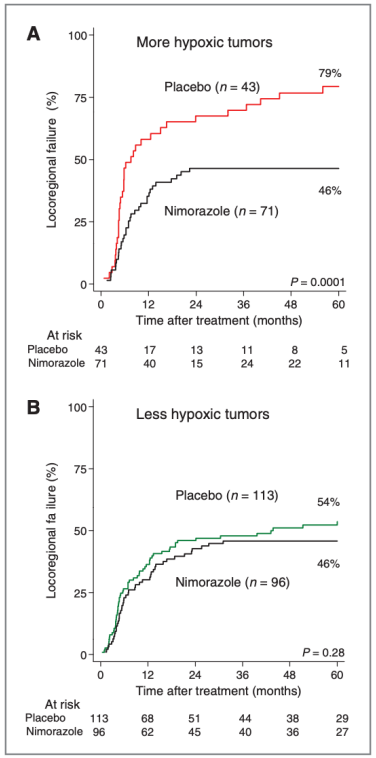
Development of a Hypoxia Gene Expression Classifier with Predictive Impact for Hypoxic Modification of Radiotherapy in Head and Neck Cancer

Kasper Toustrup[1], Brita Singers Sørensen[1], Marianne Nordsmark[1], Morten Busk[1], Carsten Wiuf[2], Jan Alsner[1], and Jens Overgaard[1]

Table 1. Hypoxia-responsive genes

In vitro derived genes	Included in hypoxia classifier	Function
ADM	ADM	Stress response
AK3L1		Nucleotide metabolism
ALDOA	ALDOA	Glucose metabolism
ANKRD37	ANKRD37	Protein–protein interactions
ARRDC3		Cell surface metabolism
BNIP3	BNIP3	Apoptosis
BNIP3L	BNIP3L	Apoptosis
C3orf28	C3orf28	Unknown
C18orf19		Unknown
CCNG2		Cell cycle regulation
EGLN1		Regulation of HIF-1 activity
EGLN3	EGLN3	Regulation of HIF-1 activity
ERO1L		Oxidoreductase
FOSL2		Cell proliferation
GPI		Glucose metabolism
HIG2		Stress response
IGFBP3		Cell proliferation
JMJD1A		Histone demethylase
KCTD11	KCTD11	Apoptosis
LOC401152		Unknown
LOX	LOX	Extracellular-matrix metabolism
NDRG1	NDRG1	Stress response
P4HA1	P4HA1	Extracellular-matrix metabolism
P4HA2	P4HA2	Extracellular-matrix metabolism
PDK1	PDK1	Energy metabolism
PFKFB3	PFKFB3	Glucose metabolism
RORA		Unknown
SLC2A1	SLC2A1	Glucose metabolism
SLC6A8		Glucose metabolism
CA9[a]		pH regulation





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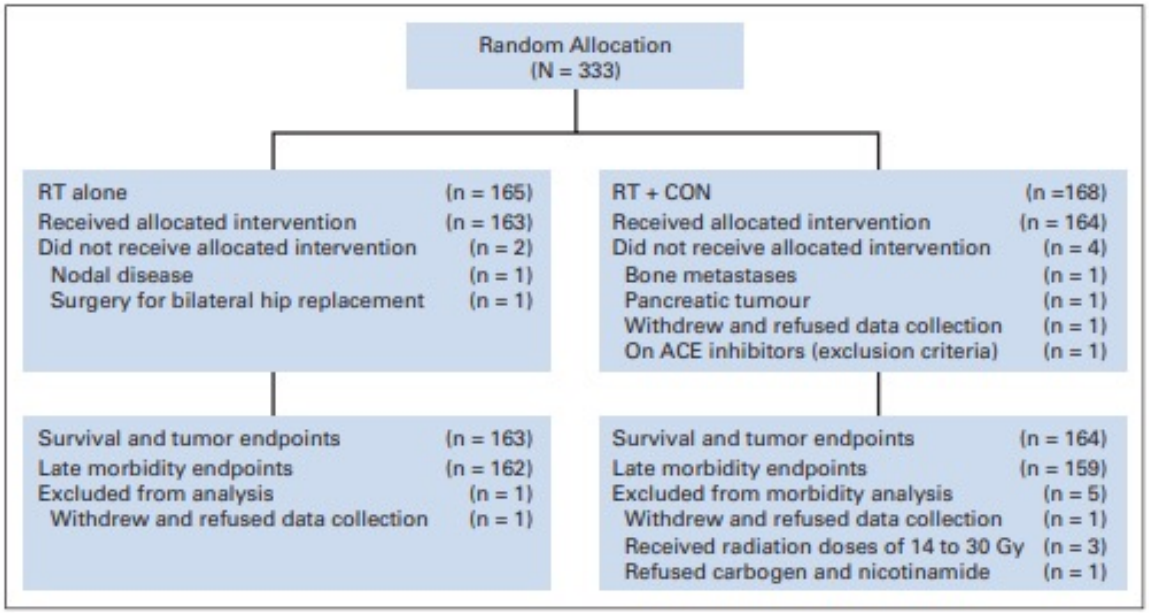
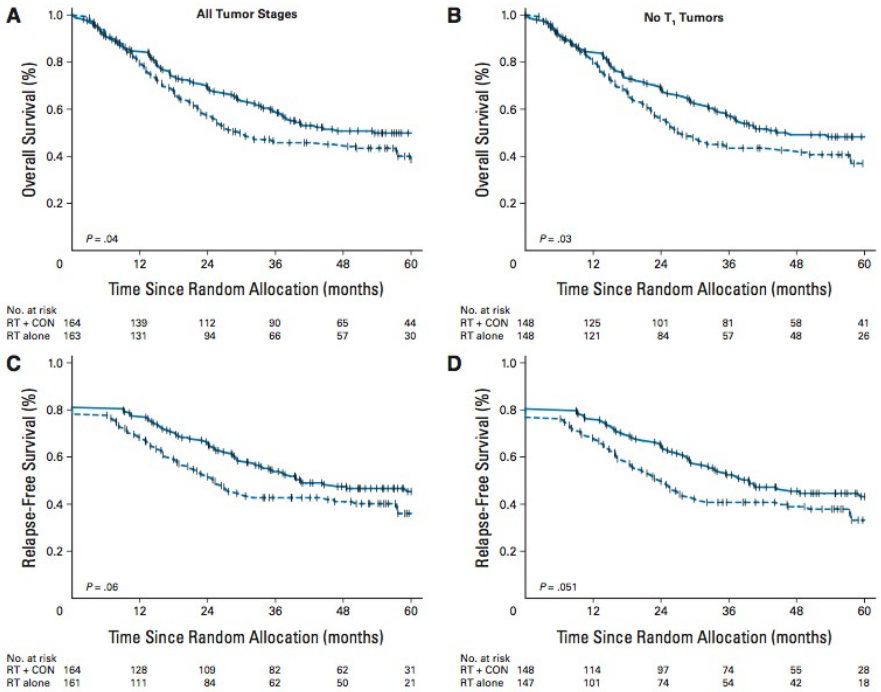
JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Radiotherapy With Concurrent Carbogen and Nicotinamide in Bladder Carcinoma

Peter J. Hoskin, Ana M. Rojas, Søren M. Bentzen, and Michele I. Saunders





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Personalized Medicine and Imaging

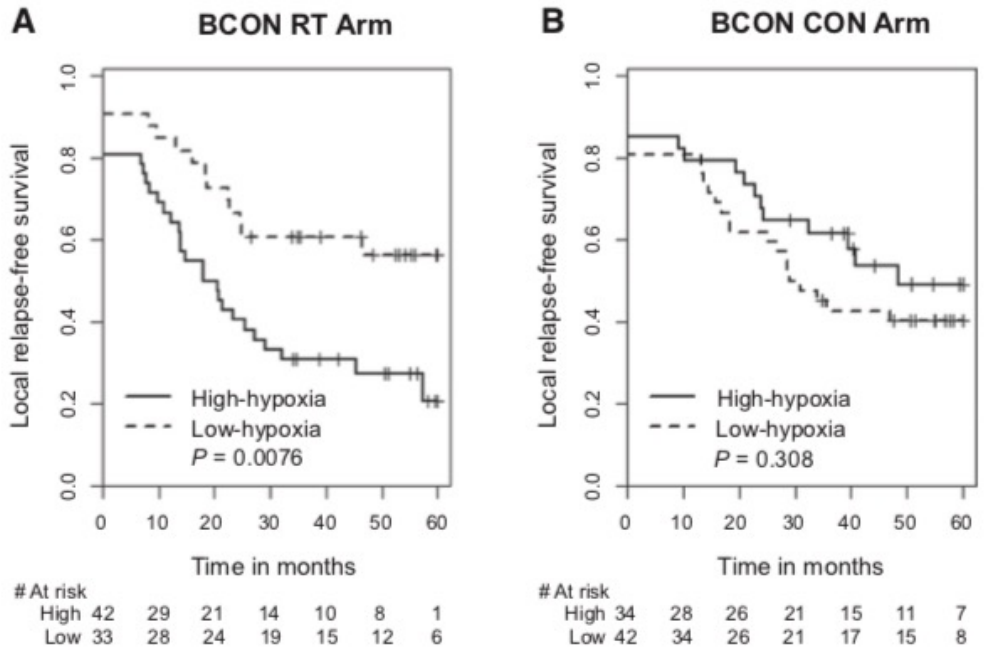
Clinical Cancer Research

A Gene Signature for Selecting Benefit from Hypoxia Modification of Radiotherapy for High-Risk Bladder Cancer Patients

Lingjian Yang[1], Janet Taylor[1,2,3], Amanda Eustace[1], Joely J. Irlam[1], Helen Denley[4], Peter J. Hoskin[5], Jan Alsner[6], Francesca M. Buffa[7], Adrian L. Harris[7], Ananya Choudhury[1], and Catharine M.L. West[1]

151 Pts, T1-T4 urothelial bladder cancer
•75 RT
•77 RT + Carbogen + Nicotinamide

A 24-gene signature was prognostic in BCON pts receiving RT alone (P=0.0076) and in RT+CON (P=0.015).



Conclusions:

A 24-gene hypoxia signature has strong and independent prognostic and predictive value for muscle-invasive bladder cancer patients. The signature can aid identification of patients likely to benefit from the addition of carbogen and nicotinamide to radiotherapy.

Conclusions

- Twenty years ago, radiation therapy and radiobiology missed the target therapy revolution.
- Around the corner is a new, more important and certainly more impactful revolution in oncology, the introduction of bioinformatics.
- The study of oxygen-guided RT is a candidate topic for the application of bioinformatics to radiotherapy and radiobiology-focused translational study planning