From basic research to clinical practice

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Translational research means different things to different people, but it seems important to almost everyone.
Translational research involves moving knowledge and discovery gained from the basic sciences to its application in clinical and community settings. This concept is often summarized by the phrases "bench-to-bedside" and "bedside-to-community" research.
• The **translational research** is the process of developing scientific discoveries into knowledge, programs and treatments that improve the health of individuals and their communities. In **basic research**, scientists study diseases in the laboratory at a molecular or cellular level.

• To improve health, findings from these basic research studies must be translated into practical applications. Translational research transforms scientific discoveries found in the laboratory into ways to prevent, diagnosis or treat disease.

• Translational research also describes the process of **moving knowledge obtained from clinical research into a wider community or practice setting**.
Translational research includes two areas of translation.

• **The first area of translation, from laboratory findings to clinical practice** (and visa versa—from clinical observations back to the laboratory for further testing) is often called “bench to bedside and back” or T1 translation. That mean that the investigators and clinicians of the future always keep in mind to improve our health through research and its application.

• **The second area of translation, to the community and back, bedside-to-community** is called T2 translation. T2 translation has long been the purview of public health scientists, who study and facilitate the application of research findings to the community.
Oxygen, life indispensable element becomes cytotoxic and destructive in form of its active metabolites (ROS)
Reactive oxygen species

- Oxygen, is a paradoxically element, in equal measure for the aerobic organisms is essential for life, but also toxic, destructive by its active metabolites. In normal state, the oxygen molecule is stable and thus less reactive, it combines directly with organic compounds (spontaneous combustion) and allows the oxidation of substances with energy role, possibly with the synthesis of ATP in respiratory chain.

- During intracellular metabolism, oxygen can undergo activation (the gain of electrons or energy gain) with the formation of highly reactive chemical species.

- Mono electronically reduction of the oxygen:
  - $O_2$ oxygen
  - $O_2^-$ superoxid
  - $H_2O_2$ hydrogen peroxide
  - $H_2O + OH.$ hydroxyl radical
  - $H_2O$
Reactive oxygen species

**Radical ROS**
- Superoxide
- Hydroxyl radical
- Nitric oxide
- Organic radical
- Peroxyl radical
- Alkoxyl radical
- Thiyl radical
- Sulphonyl radical
- Thiyl peroxyl radical

**Nonradical ROS/RNS**
- Hydrogen peroxide
- Singlet oxygen
- Ozone (trioxygen)
- Organic hydroperoxide
- Hypochlorous acid
- Peroxynitrite
The association of ROS with cancer has been difficult to understand for numerous reasons.

• 1. ROS play an important role in the initiation and progression of cancer
• 2. Cancer cells exhibit greater ROS stress than normal cells do, owing in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction
• 3. Cell-cycle progression by growth factors and receptor tyrosine kinases require ROS
• 4. Chronic inflammation, one of the major mediators of cancer, is regulated by ROS
• 5. ROS controls the expression of various tumor suppressor genes,
• 6. A high level of ROS can suppress tumor growth through the sustained activation of the cell-cycle inhibitor
• 7. Most of the chemotherapeutic and radiotherapeutic agents kill cancer cells by augmenting ROS stress

These contradictory statements imply that cancer cells die by the same mechanism which facilitates their survival. This paradox provides a great challenge for researchers whose aim is to exploit ROS stress for the development of cancer therapies.
• Over the past several years, researchers have noticed that the role of ROS depends on their level.

While a modest amount of ROS is required for tumor promotion, an excessive level serves to suppress tumors.
Sources of Biologically Relevant ROS

- The sources of ROS are both extracellular and intracellular.

<table>
<thead>
<tr>
<th>Extracellular</th>
<th>Intracellular</th>
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<tbody>
<tr>
<td>Pollutant</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Virus</td>
<td>Endoplasmatic reticulum</td>
</tr>
<tr>
<td>Tobacco</td>
<td>Peroxisome</td>
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<tr>
<td>Smoke</td>
<td>Phagosome</td>
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<tr>
<td>Diet</td>
<td>NOX</td>
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<tr>
<td>Radiation</td>
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<tr>
<td>Xenobiotic</td>
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<td>Chemo</td>
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Under normoxic conditions, intracellular levels of ROS are maintained to protect cells from damage. Scavenging of ROS is facilitated by a dedicated set of antioxidants that may be both enzymatic and nonenzymatic in nature.
Role of ROS in Tumorigenesis
Most risk factors associated with cancer interact with cells through the generation of ROS. ROS, in turn, activate the transcription factors NF-κB, activator protein-1 (AP-1), HIF-1α, signal transducer and activator of transcription 3 (STAT3), and others. These ROS-mediated transcription factors control the expression of genes involved in inflammation, cell transformation and tumor cell death or survival, proliferation, invasion, angiogenesis, and metastasis.
Role of ROS in cellular transformation

- Cellular transformation in cancer biology is a process whereby normal cells acquire properties of malignant cells. The underlying causes of malignant transformation are the gain-of-function mutations in oncogenes and the loss-of-function mutations in tumor suppressor genes. The mutations lead to perturbations of a number of signaling molecules, including p53, Raf, protein phosphatase 2A, telomerase, Ral-GEFs, phosphatidylinositol 3-kinase (PI3K), Ras, Rac, cellular v-myc myelocytomatosis viral oncogene homolog (c-Myc), STAT3, NF-κB, and HIF-1α.

Chemicals, viruses, radiation, hypoxia, and nutrient deprivation can also induce mutations in these genes, thereby giving rise to cancer cells.
RS1 hepatoma-alveolar type, used in our experiments it is a chemical induced tumor, obtained by 2-acetylaminofluorene administration in rats food. Initially obtained and transplanted by serial grafting, the tumor was an hepatocholangiomas carcinoma and these characteristics remain stable even after several passages.
Lipid peroxides production measurement

- lipid peroxidation level in the dynamic of tumoral development at tumor bearing rats. The determinations were made in serum and tumoral tissues and was expressed in percent 100% is the values of the first investigation.
• Cooper reducing activity of ceruloplasmin

Total thiol groups level in the dynamic of tumoral growth, measured in whole tumoral and liver tissues, also in serum.
The measurement of iron reducing ability made in serum and whole tumoral extract
• ROS seem to play a role in the transformation of normal cells into cancer cells. The major conclusion to be drawn is that transformed cells appear to have greater ROS levels than normal cells do. However, how ROS transform normal cells is not precisely known. Further work in this direction is needed to fully elucidate the mechanism involved in ROS-mediated malignant transformation.
Role of ROS in tumor cell death

- One of the chief characteristics of cancer cells is their inherent capacity to survive. Therefore, the major goal of cancer therapy is to selectively kill cancer cells without harming normal cells. There are three major ways by which a cancer cell can die: apoptosis, necrosis, and autophagy.
ROS and apoptosis

- Apoptosis is a tightly controlled form of cell death and can be initiated by death receptors (extrinsic pathway) or through mitochondria (intrinsic pathway). Both extrinsic and intrinsic pathways of apoptosis depend on ROS.

- In the extrinsic pathway of apoptosis, ROS are generated by Fas ligand as an upstream event for Fas activation. In turn, ROS are required for Fas phosphorylation at the tyrosine residue, which is a signal for subsequent recruitment of Fas-associated protein with death domain and caspase 8 and for apoptosis induction. In addition, ROS are required for the ubiquitination and subsequent degradation of the FLICE inhibitory protein to further enhance Fas activation.

- In contrast, the intrinsic pathway of apoptosis is characterized by the opening of the permeability transition pore complex on the mitochondrial membrane, which results in cytochrome c release, apoptosome formation, and caspase activation. Opposing effects of proapoptotic and anti-apoptotic Bcl-2 family proteins are required for opening of the permeability transition pore. ROS function to open the pore by both activating pore-destabilizing proteins (Bcl-2-associated X protein, Bcl-2 homologous antagonist/killer) and inhibiting pore-stabilizing proteins (Bcl-2 and Bcl-xL).
Extensive research over the past several years from both cell culture and animal models has demonstrated the potential of ROS in inducing apoptosis in cancer cells.

**Flow citometry measurements**

- We obtained the cell suspension by automatic disaggregation using an Medimachine, the nucleus was marked with propidium iodine in hypotonic buffer (triton X-100 0,1% and propidium iodine 50 μg/ml in sodium citrate 0.1%)
- The samples were readed by FACS Calibur flow cytometer using an Cell quest soft, and for the countering of apoptotic cells we use WinMDI programme
- For the analysis 10,000 events were aquisited.
- The hystograms type graphics represent the DNA quantity distribution and it is expressed by an function between the FL2-H fluorescence detector measurement and the total cell number.
The apoptosis rate increases during the normal tumor growth from 14.30% to 29.86%
• Apart from their ability to kill cells, ROS are also required for cancer cell survival. In fact, the ability of cancer cells to distinguish between ROS as a survival or apoptotic signal is controlled by the dosage, duration, type, and site of ROS production. However, modest levels of ROS are required for cancer cells to survive, whereas excessive levels kill them.

• ROS have dual roles: They can not only kill cancer cells but they can also promote tumor survival. The great challenge for cancer researchers is determining how to exploit this dual property of ROS for therapeutic development.
Role of ROS in tumor cell proliferation

- A precise set of cell cycle regulators such as cyclins and cyclin-dependent kinases (CDKs) control the progression of cell-cycle events. CDK activity is controlled by the opposing effects of cyclins and CDK inhibitors. CDK inhibitors negatively regulate CDK activity, whereas cyclins are required for CDK activity and cell cycle progression. Another protein, c-Myc, is required for the G1-to-S-phase transition. The expression of c-Myc, in turn, is regulated by cdc25, a phosphatase that activates CDKs.
Intracellular ROS produced by exogenous stimuli as well as exogenous administration of ROS have been shown to enhance the proliferation of numerous cancer types

• For example, in experimental models, exogenous administration of H$_2$O$_2$ was shown to enhance the proliferation of hepatoma cells by increasing protein kinase B and extracellular signal-regulated kinase (ERK) activities.
• ROS produced by low concentrations of arsenite has been shown to enhance the proliferation of breast cancer cells by recruiting cells into the S phase of the cell cycle, enhancing the expression of c-Myc and heme oxygenase-1, and increasing NF-κB activity.


• The role of ROS in promoting tumor proliferation is further supported by observations that agents with the potential to inhibit ROS generation can also inhibit tumor cell proliferation.

• Curcumin has been shown to inhibit the proliferation of lymphoma cells by increasing endogenous antioxidant enzyme activity and by inhibiting NF-κB activity.

Inhibition of ROS generation by N-acetyl-L-cysteine (NAC), one of the most widely used ROS scavengers, has been correlated with decreased proliferation of cancer cells. For example, treatment of glioma cells with NAC inhibited cell proliferation by arresting cells in the G1 phase; this inhibition was correlated with a decrease in the activities of AKT, ERK1/2, and NF-jB

Role of ROS in tumor cell invasion, angiogenesis, and metastasis

• Tumor cell invasion, angiogenesis, and metastasis are interrelated processes that represent the final, most devastating stage of malignancy. The process involves cell growth, adhesion, and migration, proteolytic degradation of tissue barriers and formation of new blood vessels.

• Accumulating evidence over the past several years from both in vitro and in vivo studies has indicated a role for ROS as a signaling mediator of angiogenesis and metastasis. ROS has been shown to mediate these effects through induction of transcription factors and genes involved in angiogenesis and metastasis. However, the role of ROS in modulating tumor cell metastasis and angiogenesis has seemed paradoxical: High ROS levels suppress tumor angiogenesis and metastasis by destroying cancer cells, whereas sub-optimal concentrations assist cancer cells in metastasizing.
• **Treatment** with Avastin was performed once by every week during 3 month, 5 mg/kg body weight

• **Biological materials:** tumoral and liver tissues was removed after 3, 5, 10 and 15 weeks of Avastin administration, obtained from tumor bearing rats in a control and in a treated group.

• **Biochemical assays:** lipid peroxides were measured with TBARS reaction, total –SH groups was monitorised by Schosinsky assay, total antioxidant activity was measured by the ability of biological sample to reduce the iron (FRAS) method involving the Fe$^{III}$ –TPTZ Fe$^{II}$ –TPTZ reaction.

• All the assays were performed on crude tissue homogenate
Lipid peroxides and total –SH group level during the anti-VEGF treatment in tumoral and liver tissue.
Total antioxidant activity during the anti-VEGF treatment in tumoral and liver tissue
• Interestingly in one study, modulation of lung cancer metastasis was dependent on ROS type. The hydroxyl radical upregulated caveolin-1 expression and promoted metastasis, whereas superoxide and H2O2 down-regulated caveolin-1 and inhibited metastasis

Role of ROS in cancer therapy

**Chemotherapy**

- The cancer drugs approved by the U.S. Food and Drug Administration may be basically classified into two categories: **nontargeted and targeted**. The nontargeted drugs may be cell-cycle specific or cell-cycle nonspecific. Targeted cancer drugs block the growth and spread of cancer by interfering with signaling molecules, growth factors, and receptors associated with tumor growth and progression. Some of these targeted drugs are monoclonal antibodies such as rituximab, ibritumomab tiuxetan, ofatumumab, and alemtuzumab.

- Procarbazine was one of the first drugs developed based on its ROS-generating properties. Procarbazine undergoes oxidation in aqueous solution and results in H\text{2}O\text{2} production that is believed to be essential for the cytotoxic effects of the drug.

- Some compounds have exhibited anticancer activity in clinical trials through ROS generation, but their mechanism of ROS production is unknown.

- Anticancer agents have also been shown to enhance ROS stress in cancer cells by inhibiting the antioxidant defense system.

- Some of the anticancer agents target the GSH system.
A List of U.S. Food and Drug Administration-Approved Targeted Anticancer Drugs That Work Through Generation of Reactive Oxygen Species

<table>
<thead>
<tr>
<th>Target Drug</th>
<th>Year</th>
<th>Cancer type</th>
</tr>
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<tbody>
<tr>
<td>Rituximab</td>
<td>1997</td>
<td>Non-Hodgkin’s lymphoma, CLL</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan</td>
<td>2002</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Tositumomab</td>
<td>2003</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>2009</td>
<td>CLL</td>
</tr>
<tr>
<td>CD 33Y Gemtuzumab ozagamicin</td>
<td>2000</td>
<td>Acute myelogenous leukemia</td>
</tr>
<tr>
<td>CD 52Y Alemtuzumab</td>
<td>2001</td>
<td>CLL</td>
</tr>
<tr>
<td>CD 117Y Imatinib</td>
<td>2001</td>
<td>Gastrointestinal, CML</td>
</tr>
<tr>
<td>Interleukin-2 Y Aldesleukin</td>
<td>1998</td>
<td>Melanoma, Renal</td>
</tr>
<tr>
<td>Denileukin diftitox</td>
<td>1999</td>
<td>Cutaneous T-cell lymphoma</td>
</tr>
<tr>
<td>EGFRY Gefitinib</td>
<td>2003</td>
<td>Lung</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>2004</td>
<td>Colorectal, Head and neck</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>2004</td>
<td>Prostate, Lung</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>2006</td>
<td>Colorectal</td>
</tr>
<tr>
<td>HER2 /neuY Trastuzumab</td>
<td>2010</td>
<td>Breast, Gastric</td>
</tr>
<tr>
<td>VEGFRY Bevacizumab</td>
<td>2004</td>
<td>Colorectal, Renal, Lung, Glioblastoma</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>2009</td>
<td>Renal</td>
</tr>
<tr>
<td>HER2 and EGFRY Lapatinib ditosylate</td>
<td>2007</td>
<td>Breast</td>
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<tr>
<td>EGFR and VEGFRY Vandetanib</td>
<td>2011</td>
<td>Thyroid</td>
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<tr>
<td>PDGFR, VEGFR and Sorafenib tosylate</td>
<td>2005</td>
<td>Renal, Liver</td>
</tr>
<tr>
<td>Retinoid X receptor [ Bexarotene</td>
<td>2000</td>
<td>Cutaneous T-cell lymphoma</td>
</tr>
<tr>
<td>Drug Name</td>
<td>Year</td>
<td>Indications</td>
</tr>
<tr>
<td>-----------</td>
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</tr>
<tr>
<td>CD 117Y Sunitinib malate</td>
<td>2006</td>
<td>Renal, Gastrointestinal</td>
</tr>
<tr>
<td>PDGFR, BCR-ABL and CD 117Y Nilotinib</td>
<td>2007</td>
<td>CML</td>
</tr>
<tr>
<td>PDGFR, BCR-ABL, Src and CD 117Y Dasatinib</td>
<td>2006</td>
<td>CML, ALL</td>
</tr>
<tr>
<td>RANKLY Denosumab</td>
<td>2010</td>
<td>MM, Bone</td>
</tr>
<tr>
<td>HDACY Vorinostat</td>
<td>2006</td>
<td>Cutaneous T-cell lymphoma</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>2009</td>
<td>Cutaneous T-cell lymphoma</td>
</tr>
<tr>
<td>mTORY Temsirolimus</td>
<td>2007</td>
<td>Renal</td>
</tr>
<tr>
<td>Everolimus</td>
<td>2009</td>
<td>Renal, Astrocytoma</td>
</tr>
<tr>
<td>ProteasomeY Bortezomib</td>
<td>2003</td>
<td>Mantle cell lymphoma, MM</td>
</tr>
<tr>
<td>CTLA 4Y Ipilimumab</td>
<td>2011</td>
<td>Melanoma</td>
</tr>
<tr>
<td>CXCR4Y Plerixafor acetate</td>
<td>2008</td>
<td>Non-Hodgkin’s lymphoma, MM</td>
</tr>
<tr>
<td>GnRH[ Leuprolide acetate</td>
<td>2000</td>
<td>Prostate</td>
</tr>
<tr>
<td>GnRHY Abarelix</td>
<td>2003</td>
<td>Prostate</td>
</tr>
<tr>
<td>Degarelix</td>
<td>2009</td>
<td>Prostate</td>
</tr>
<tr>
<td>AromataseY Anastrozole</td>
<td>1996</td>
<td>Breast</td>
</tr>
<tr>
<td>Exemestane</td>
<td>1999</td>
<td>Breast</td>
</tr>
<tr>
<td>Letrozole</td>
<td>2001</td>
<td>Breast</td>
</tr>
<tr>
<td>Estrogen receptorY Tamoxifen citrate</td>
<td>1977</td>
<td>Breast</td>
</tr>
<tr>
<td>SERM Toremifene</td>
<td>1997</td>
<td>Breast</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>2007</td>
<td>Breast</td>
</tr>
<tr>
<td>SERD Fulvestrant</td>
<td>2002</td>
<td>Breast</td>
</tr>
</tbody>
</table>
Radiotherapy

• Similar to chemotherapy, radiotherapy employs ROS to eradicate cancer cells. Radiotherapy uses X-rays, c-rays, and, to a lesser extent, heavy particle radiation, such as with protons and neutrons. Radiation kills cancer cells by inducing apoptosis and mitotic failure and by inhibiting their proliferation.

• The role of ROS in mediating radiation-induced cancer cell killing is evident from a number of preclinical and clinical studies.
Cells irradiated with Co-60 at Theratron 100E radiation beam 11x11 cm². remote source irradiated surface environment 95 cm irradiation depth 5 cm The dose 1.5-5 Gy irradiation time 2,62 (depending on the work load)

Irradiation times: 20, 40, 60 minutes
The lipid peroxides increase during the irradiations.

Cu-oxidase activity of ceruloplasmin
Total –SH groups

- Normal
- 20 min irrd
- 40 min irrd
- 60 min irrd

Total antioxidant capacity

- Normal
- 20 min irrd
- 40 min irrd
- 60 min irrd
• Type I behavior of oxidative stress

- Increased lipid peroxides and activation of antioxidant systems, both types of graphics are on the rise
Type II behavior of oxidative stress

Lipid peroxides increase and decrease endogenous antioxidant activity - metabolic type that is associated with an increased risk of complications SIRS.
Type III behavior of oxidative stress

Peroxidation and antioxidant reactions are down - this is associated with a poor prognosis and the effectiveness of radiotherapy treatment can be given tumor type anaerobic
• Type IV behavior of oxidative stress

Decrease lipid peroxides as a measure of oxidative reactions initiated by radiation and increase antioxidant protection due to activation of natural systems, this type is associated with immune reactivity and decreased hipo-inflammation, representing a new risk group.
Role of ROS in Eliminating Chemoresistance and Radioresistance

• One of the major problems in treating cancer is that tumor cells, although initially sensitive, gradually develop resistance to chemotherapy and radiotherapy, in part owing to the induction of multidrug resistance proteins.

• Extensive research over the past several years has indicated that ROS-generating anticancer agents can reduce the chemoresistance and radioresistance of cancer cells.

• In this regard, nutraceuticals have shown promise in sensitizing tumor cells to chemotherapeutic and radiotherapeutic agents.

• Curcumin has been shown to eliminate chemoresistant cells by sensitizing them to chemotherapy, in part by inhibiting pathways that lead to treatment resistance.
• For example, curcumin treatment in conjunction with 5-fluorouracil (5-FU) or with both 5-FU and oxaliplatin resulted insignificantly greater growth inhibition and more apoptosis in HCT116 and HT29 colon cancer cells than that caused by curcumin alone or 5-FU alone.

• In another study, curcumin given with tamoxifen resulted in synergistically induced apoptosis and autophagy in chemoresistant melanoma cells that correlated with an increase in ROS generation.

• An interesting finding from that study was that noncancerous cells were unaffected by the combination treatment.

• A number of other in vitro and in vivo studies have provided evidence for curcumin’s use singly or as an adjunct to current chemotherapeutic drugs.

• Other nutraceuticals have demonstrated usefulness in reducing tumor cell resistance to chemotherapy or radiotherapy.
Summary, Conclusion, and Future Perspectives

ROS are integral components of cell signaling pathways and have been shown to regulate cell transformation, survival, proliferation, invasion, angiogenesis, and metastasis. Paradoxically, ROS can also suppress tumor progression, and most chemotherapeutic and radiotherapeutic agents work by augmenting ROS stress in cancer cells.

Due to the dual role of ROS, both pro-oxidant- and antioxidant-based anticancer agents have been developed. However, modulation of ROS signaling alone seems not to be an ideal approach, because some cancers are highly adapted to ROS stress, the redundant pathways supporting cancer growth are complex, and some ROS-generating anticancer drugs are associated with toxic side effects. Combinations of ROS-generating agents with agents that can break the redox adaptation could be a better strategy for enhancing cancer cell cytotoxicity.

Due to their ROS-generating and multi-targeting properties, nutraceuticals might offer an advantage in selectively killing cancer cells. However, only a limited number of nutraceuticals have shown clinical efficacy, and none has been approved for human use. Future attempts in this direction will hopefully lead to the development of novel drugs.
Thank you!