7.341 New Ammunition for the War Against Cancer: Personalized Therapeutic Regimens based on Defective DNA Damage Signaling in Tumors

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Fall 2008. Wednesdays, 3 pm - 5 pm. Room 68-151. Class time is flexible.

Prerequisites:

The prerequisites for this course are 7.03, 7.05, 7.06, or 7.28. Grading: Pass/Fail.

Course summary:

In 1971, President Nixon declared the "War on cancer". Over recent years we have seen the development of new armament for this war - treatment strategies that are based on unique characteristics of tumor cells. An increased propensity for mutation is a hallmark of cancer, and an increasing number of genes involved in the DNA damage response and DNA repair have been found mutated or inactivated in human cancers, thus underlining the importance of an intact DNA damage response as a critical anti-cancer barrier. Cellular responses to DNA damage constitute one of the most important fields in cancer biology. Exciting work in this area has taught us important lessons, such as: DNA damage can cause cancer; paradoxically, the induction of DNA damage is also the mechanism of action of many currently used anti-cancer therapeutics, such as radiation and chemotherapy; and DNA damage of normal tissues is responsible for most of the side effects of cancer therapy, such as hair loss. In this class we will analyze classical and recent papers from the primary research literature to gain a profound understanding of cell cycle regulation and DNA damage checkpoints that act as powerful emergency brakes to prevent cancer. We will consider basic principles of cell proliferation and molecular details of the DNA damage response and we will discuss the methods and model organisms typically used in this field. Building on this foundation we will explore new concepts in the treatment of cancer that are based on and exploit characteristic differences in the DNA damage response between normal cells and cancer cells. While mutations in genes involved in cell cycle control and the DNA damage response allow the runaway proliferation of incipient cancer cells, it can also be seen as the "Achilles heel" of cancer. We will see that therapeutic regimens emerge that are guided by a spectrum of characteristic mutations that differ between individual patients – paving the way for personalized anti-cancer therapy. This course will not stop at discussing the research literature. We will go one step further by gathering and analyzing real data in an MIT Cancer Biology laboratory. Refreshments will be provided.

Course Objectives:

One primary objective of this course is to introduce students to the analysis of primary research literature. To achieve this goal, we will have weekly sessions, during which we will engage in a detailed discussion of two scientific papers. Our readings will include both classic and recent breakthrough papers. With the exception of the first meeting, no lectures will be given. Instead, course participants will actively present as well as drive the discussions of the papers.

The second objective of this course is for students to gain a deeper understanding of the response mechanisms a cell puts into place when it faces DNA damage. We will learn that these defense mechanisms are crucial for cellular survival and that misregulation of these processes can lead not only to cell death but also to increased mutation frequency, thus paving the way for cancer development. The cellular mechanisms involved in the so-called DNA damage response are well-studied both at the organism level as well as at the molecular level. We will see that the core machinery of the DNA damage response consists of a highly specialized network of protein kinases and phosphatases, which are rapidly acting enzymes that can add or remove phosphates to substrates. Many different components of this cellular defense mechanism have been found mutated in different cancers. This impairment of DNA quality control and repair mechanisms allows the accumulation of mutations and the development of cancer. Over recent years we have seen the development of therapeutic regimens that are based on the impaired DNA damage response of cancer cells. During this class we will use our understanding of basic cell cycle regulatory mechanisms to understand how these new therapeutic concepts work and why they are specifically targeting cancer cells.

Course requirements:

This course is graded pass/fail. The grading will be based on participation during presentations and discussions as well as on two assignments. Attendance at all meetings is very important. If an absence is unavoidable, instructors should be notified in advance and make-up work will be assigned.

Assignments:

Mid-term assignments:

This assignment will consist of writing an abstract for a research article. Students will be given a part of a research article (results, materials and methods and the figures). With these core parts of the research article, each student should write a comprehensive 250-word abstract that summarizes the results of the key experiments and the main conclusions. The abstract should aim to put the specific findings in a more general perspective, as well.

Final Assignment:

Each student will be asked to prepare an oral presentation based on a research article that elucidates the function of a gene involved in the DNA damage response. The research articles will focus on genes implicated in cancer or cancer-prone syndromes. Students will be asked to describe how the clinical features of this syndrome could be explained by the underlying defect. Students will discuss the key figures from the article and also put the findings of the paper into a broader perspective concerning the associated disease.
The presentation will be given to the entire group. Students will be encouraged to actively participate in a discussion following each presentation.

Course program:

Week	Topics	Overview	Reading
1 (09/03/08)	**Introduction**	At the first meeting, we will briefly outline the **general requirements**, the **class schedule** and **administrative details**. The instructor and students will introduce themselves. The instructor will then summarize basic aspects of cell cycle regulation and the **DNA damage response network**. We will introduce the different DNA damage checkpoints that evolved to protect cellular DNA from various sources of damaging agents with a special focus on differences between normal cells and cancer cells. Also, commonly used **model systems** and **standard techniques** will be introduced and discussed.	
2 (09/10/08)	**The cell cycle**	We will discuss the **basic cell cycle machinery**, using two classic papers describing the principles of cellular proliferation. We will learn about the **different stages of the cell cycle** and understand their specific characteristics. During this session we will also discuss the molecular mechanisms that make cell cycle progression dependent on **extracellular cues** (such as growth factors). We will discuss mechanisms by which cancer cells can acquire the ability for **autonomous growth**.	Pines J and Hunter T *Isolation of a human cyclin cDNA: "evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34cdc2."* Cell (1989) 8;58(5):833-46 Simanis V, Nurse P. *The cell cycle control gene cdc2+ of fission yeast encodes a protein kinase potentially regulated by phosphorylation.* Cell (1986) 45(2):261-8.

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3 (09/17/08)	**Cell cycle control – the role of the tumor suppressor pRb in the G_1/S transition**	It is essential that the different phases of the cell cycle are precisely coordinated and controlled so that one phase is completed before the next one can begin. **Errors in coordination can lead to chromosomal aberrations**-- chromosomes or parts of chromosomes can be lost, rearranged, or distributed unequally between the daughter cells. This type of alteration is often seen in cancer cells. A profound understanding of (1) how cells determine when and how to multiply and of (2) how that process can go awry is fundamental for understanding the **development of cancer**. Furthermore, this knowledge is essential to enable scientists and clinicians to predict, prevent, or reverse a tumor's growth properties. This week we will discuss how cell cycle control genes were first identified using yeast as a model organism. We will also discuss the role of tumor suppressor genes in the control of cell cycle progression.	Hartwell, L. H., J. Culotti, and B. Reid. "Genetic control of the cell-division cycle in yeast. I. Detection of mutants." Proc. Natl Acad. Sci USA 66 (1970): 352-359. Goodrich, D. W., N. P. Wang, Y. W. Qian, E. Lee, and W. H. Lee. "The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle." Cell 67 (1991): 293-302.
4 (09/24/08)	**Cdk-regulation**	We will discuss how the core cell cycle machinery is subject to regulation. Specifically, we will discuss the **regulation of Cyclin-dependent kinases** (the major driving forces of the cell cycle) by **phosphorylation and dephosphorylation**.	Parker LL and Piwnica-Worms H. *"Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase."* Science (1992) 257, 5078, 1955-1957 Gautier J, Solomon MJ, Booher RN, Bazan JF and Kirschner MW. *"Cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2."* Cell (1991) 4;67(1):197-211.

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5 (10/01/08)	**Checkpoint control of the cell cycle**	We will discuss the multiple means by which the **DNA damage-activated checkpoints** regulate cell cycle progression in response to DNA damage. Both **transcriptional and post-translational responses** will be discussed using two landmark papers.	Matsuoka S, Huang M, Elledge SJ. *"Linkage of ATM to cell cycle regulation by the Chk2 protein kinase."* Science. (1998) 4;282(5395):1893-7. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. *"Radiation-induced cell cycle arrest compromised by p21 deficiency"* Nature (1995) 12;377(6549):552-7.
6 (10/08/08)	**p53 Regulation**	One of the most commonly mutated genes in human cancer is **p53**. We will discuss the important role of the prominent **tumor suppressor gene** p53 in the cellular responses to DNA damage. We will see that p53 has a dual role in the response to DNA damage. On one hand it can mediate **cellular survival**, by arresting the cell cycle and inducing the expression of genes involved in DNA repair. On the other hand p53 is a potent inducer of programmed cell death – **apoptosis**. We will discuss potential **therapeutic strategies** that build on the common loss of p53 in human malignancies.	Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW. *"DNA Damage-Induced Activation of p53 by the Checkpoint Kinase Chk2."* Science (2000) 287(5459):1824-7 Honda R and Yasuda H. *"Association of p19ARF with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53."* The EMBO Journal (1999) 18, 22–27

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7 (10/15/08)	**The DNA damage checkpoint differs depending on cell cycle stage**	We will dive deeper into the core mechanisms that control the **cellular response to DNA damage**. We will discuss different modes of regulation of the **phosphatase Cdc25**. Furthermore, we will analyze different types of repair strategies that are dependent on the cellular DNA content and thus on the cell cycle stage.	Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, Jackson SP. "*ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks."* Nature Cell Biology. (2006) Jan;8(1):37-45 Jin J, Shirogane J , Xu L, Nalepa G, Qin J, Elledge SJ and Harper JW. *"SCFb-TRCP links Chk1 signaling to degradation of the Cdc25A protein phosphatase."* Genes & Develpment (2003) 17:3062-3074
8 (10/22/08)	**'To die or not to die' – the decision between repair and apoptosis**	We will discuss two different possible outcomes after exposure to genotoxic agents, controlled by **p53**. We will use two papers to show that both **repair** of the damaged genome and activation of **programmed cell death** can be initiated by similar signaling pathways.	Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. *"PUMA induces the rapid apoptosis of colorectal cancer cells."* Molecular Cell (2001);7(3):673-82. Adimoolam S, Ford JM. *"p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene."* Proc Natl Acad Sci U S A. (2002) 1;99(20):12985-90

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9 (10/29/08)	**Structural insights into the DNA damage response**	We will discuss **structural insights** concerning the molecular machinery that controls the DNA damage response. We will focus on **Rad50**, a member of the **MRN complex**, as well as **MDC1** and its interaction partner **H2AX**. These proteins are involved in the very early stages of the DNA damage response and together form the structures that serve as an anchoring platform for downstream signaling molecules.	Moreno-Herrero F, de Jager M, Dekker NH, Kanaar R, Wyman C, Dekker C. *"Mesoscale conformational changes in the DNA-repair complex Rad50/ Mre11/Nbs1 upon binding DNA."* Nature (2005) 437(7057):440-3. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. *"MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks."* Cell (2005) 123(7):1213-26.
10 (11/05/08)	**Mid-term assignment due**	**Field trip: visit to an MIT Biology Laboratory** Now that have gathered a solid background concerning checkpoint signaling, we will be introduced to the real **methods**. We will visit a **molecular biology laboratory** focused on studying DNA damage and the subsequent checkpoint response. Typical **checkpoint assays** will be demonstrated. We will examine different samples of untreated or damaged material that have been analyzed by **FACS** (Fluorescence Activated Cell Sorter) and by **high-resolution fluorescence microscopy**. We will analyze our data using the knowledge acquired in our prior sessions.	

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11 (11/12/08)	**Defective DNA damage responses and cancer**	We will learn about the importance of the DNA damage response by studying the impact of checkpoint failure due to mutations in critical checkpoint genes. We will discuss the consequences of loss of **Chk1** and **p53**. Both papers use the **mouse** as a model organism.	Lam MH, Liu Q, Elledge SJ, Rosen JM. *"Chk1 is haploinsufficient for multiple functions critical to tumor suppression."* Cancer Cell (2004) 6(1):45-59 Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. *"Restoration of p53 function leads to tumour regression in vivo."* Nature (2007) 8;445(7128):661-5.
12 (11/19/08)	**Checkpoint-related syndromes**	It is perhaps not surprising that genes involved in the DNA damage response and DNA repair are commonly mutated in incipient **cancer cells** on their road to malignancy. Defects in the ability to detect and repair mutations increase the likelihood of acquiring mutations that will ultimately help to fuel the runaway proliferation of cancer cells. Defects in **checkpoint signaling** are associated with a number of human diseases. Besides **spontaneous mutations** in checkpoint genes, **familial syndromes** have been identified in which specific checkpoint genes are mutated. During this session, two such syndromes will be discussed and compared.	Gilad S, Chessa L, Khosravi R, Russell P, Galanty Y, Piane M, Gatti RA, Jorgensen TJ, Shiloh Y, Bar-Shira A. *"Genotype-phenotype relationships in ataxia-telangiectasia and variants"*. American Journal of Human Genetics. (1998) 62(3) 551-61. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA. *"Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome"* Science (1999) (5449):2528-31.

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13 (11/26/08)	**Cancer treatment based on knowledge about the DNA damage response – targeted therapeutics**	We will discuss two recent papers that make use of our increasing understanding of DNA damage signaling to derive new **therapeutic strategies** that specifically target **cancer cells** rather than healthy cells. We will learn about the concept of **personalized cancer therapy**.	Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A. *"Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy."* Nature (2005) 14;434(7035):917-21. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. *"In vivo activation of the p53 pathway by small-molecule antagonists of MDM2."* Science (2004) 6;303(5659):844
14 (12/03/08)	**Cancer treatment based on specific mutations that "drive" malignant growth – exploiting oncogene addiction**	We will discuss two **success stories** of **targeted anti-cancer therapy**. Our knowledge about general principles of cancer cell proliferation will serve as a foundation to understand the mechanism of action of two recently developed **anti-cancer therapeutics**. We will discuss why these drugs specifically target cancer cells. Unfortunately, clinicians have seen the development of new **resistance mechanisms** that can abolish the beneficial effects of these drugs. We will discuss different resistance mechanisms and think about strategies that help to avoid the problem of resistance.	Druker, B. J., S. Tamura, E. Buchdunger, S. Ohno, G. M. Segal, S. Fanning, J. Zimmerman, and N. B. Lydon. "Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells." Nature Med. 2 (1996): 561-566. Paez, J. G., P. A. Janne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson, and M. Meyerson. "EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy." Science 304 (2004): 1497-1500.

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15 (12/10/08)	**Final assignments due**	For the **final presentations** each student will be assigned a research article that describes the function of a critical checkpoint protein related to a **human disease**. Students will discuss the **key figures** and summarize the **molecular functions** of the protein described in the paper. Furthermore, the students will briefly discuss the function of this protein within the global DNA damage network that was discussed throughout the course. Lastly, the students will be asked to explain the **clinical features** of the related **human disease** (mentioned in the research article), based on the molecular details that were described in the paper. The presentations are scheduled for **15 minutes** (10 minute talk [7-8 powerpoint slides], followed by a **5 minute discussion**). The presentation will be given to the entire group. Students will be encouraged to **actively participate** in the discussion following each presentation.	