"Oncogenic Shock": Explaining Oncogene Addiction through Differential Signal Attenuation

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Abstract "Oncogene addiction" describes the curious acquired dependence of tumor cells on an activated oncogene for their survival and/or proliferation, a phenomenon that has important implications for the success of targeted cancer therapies. However, the mechanisms explaining oncogene addiction remain elusive. We propose that "addiction" may be an illusion generated as a consequence of differential attenuation rates of prosurvival and proapoptotic signals emanating from an oncoprotein acutely following its inactivation. According to this model, which we call "oncogenic shock," prosurvival signals dissipate quickly on oncoprotein inactivation whereas proapoptotic signals linger sufficiently long to commit the cell to an apoptotic death. This mechanism may contribute to the rapid and dramatic clinical responses observed in some cancer patients treated with selective tyrosine kinase inhibitors and could yield additional drug targets that determine the balance of signaling outputs from activated oncoproteins.

The term "oncogene addiction" was first coined by Bernard Weinstein to describe an intriguing phenomenon in which tumor cells, despite the accumulation of multiple genetic alterations, seem to become dependent on a single oncogenic activity for their sustained proliferation and/or survival (1). This phenomenon has now been observed in multiple experimental settings, including various cell culture and transgenic animal models. In these studies, the conserved theme is that tumor cells expressing an activated oncogene tend to undergo rapid apoptosis, or sometimes growth arrest and differentiation, upon acute disruption of the oncogenic activity (via antisense/RNA interference–mediated suppression of oncogene expression, switching off of an inducible oncogene, or small-molecule inhibitors that disrupt the activity of an oncoprotein). Oncogene addiction has also been proposed as a likely explanation for the rapid regression of human tumors, which is sometimes seen in patients treated with various targeted molecular cancer therapies, such as the selective tyrosine kinase inhibitors imatinib (Gleevec) and gefitinib (Iressa), highlighting potentially important implications of this phenomenon in the treatment of cancer (2).

At first glance, this may seem trivial—that an oncogene that has clearly contributed to the tumorigenic process is also required to maintain the malignant phenotype is not itself surprising. However, the curious nature of this phenomenon is highlighted by the fact that cancer cells can undergo massive apoptosis in response to inactivation of a pathway of which the disruption in noncancerous cells has no obvious consequences. For the purposes of this discussion, we specifically consider oncogene addiction as the phenomenon in which oncogenically transformed cells rapidly undergo apoptosis following the selective disruption or inactivation of the oncogene or its protein product.

To explain oncogene addiction, it has been suggested that the intricate signaling circuitry of a tumor cell may be profoundly and irrevocably altered by the presence of the oncogenic activity, and that one consequence of this cellular reprogramming is the development of a strict dependence on expression of the oncogene (1, 3, 4). Such reprogramming almost certainly occurs, and its influence on the balance of signals that lead to proliferation, differentiation, senescence, and apoptosis is likely to contribute to tumorigenesis. However, the molecular mechanisms underlying such reprogramming as it relates to drug-induced apoptosis remain unclear.

An Alternative Hypothesis to Explain Oncogene Addiction

Here, we present an alternative hypothesis to explain the phenomenon of oncogene addiction. We hypothesize that tumor cells are not dependent per se on any one oncogenic activity. Rather, we suggest that the apoptotic response observed in tumor cells upon acute disruption of an oncogene product results from differential decay rates of the various multiple prosurvival and proapoptotic signals emanating from the oncoprotein following its inactivation (Fig. 1). The downstream prosurvival signals are relatively short-lived following oncogene inactivation when compared with the longer-lived apoptotic signals, which persist for a sufficient period of time to drive the tumor cell down an irreversible pathway of apoptotic death. In fact, the so-called "commitment phase" of the apoptotic process has been shown to be as short as a few hours (5), and if left unchecked by a counteracting survival

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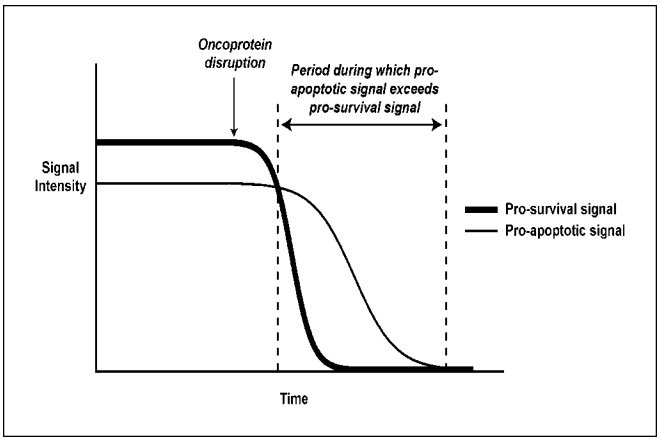

Fig. 1. A schematic illustration of the role of differential signal attenuation in "oncogene addiction." The model proposes that prosurvival and proapoptotic signals emanating from an active oncoprotein in a tumor cell are normally balanced such that the survival output predominates. However, on acute disruption of oncogene function, prosurvival signals dissipate very rapidly whereas proapoptotic signals linger sufficiently long such that the cell becomes committed to an apoptotic death. "Signal intensity" is an arbitrary value that describes the relative strength of prosurvival and proapoptotic signals emanating from the active oncoprotein.

signal, this temporary imbalance in the signaling output from an oncoprotein should be sufficient to drive an apoptotic outcome. We refer to this mechanism as "oncogenic shock." In contrast to other models of oncogene addiction in which a cancer cell passively defaults to apoptosis following withdrawal of an oncogenic signal, in this scenario, a lingering proapoptotic output from the oncoprotein is actively required to promote cell killing. Consistent with the view that an active proapoptotic output from an oncoprotein must be produced to result in apoptosis following signal attenuation, we have observed that lung cancer cells treated with the epidermal growth factor receptor (EGFR) inhibitor gefitinib are more efficiently killed when the medium is supplemented with the receptor ligand, EGF. This and related findings described below have led us to conclude that experimental studies that have led to the oncogene addiction concept may have inadvertently produced an illusion of oncogene dependence.

Accumulating evidence indicates that most, if not all, of the well-studied oncoproteins, including Ras, BCR-ABL, EGFR, and Src, are each able to transduce antiapoptotic as well as proapoptotic signals via their interactions with multiple downstream effectors, often arranged in complex signaling cascades. In normal cells, such signaling duality is probably a mechanism that allows for rapid switching between biological outputs from such proteins. In the context of a tumor cell, the balance between oncoprotein-induced survival signaling and apoptotic signaling clearly favors a survival outcome, although tumor cell lines and tumor tissues typically exhibit a relatively high rate of apoptotic cell death, indicating that cancer cells are probably teetering on the threshold of an apoptotic event. This suggests that multiple proapoptotic and prosurvival signals emanating from an active oncoprotein neutralize each other to maintain a net survival state, and it is easy to imagine how such a balance could shift dramatically during the first few hours following inactivation of the signal source, particularly if each of those multiple downstream signals dissipates at a different rate. It has recently been similarly suggested that a mechanism involving differential decay of promitogenic and proapoptotic signals might contribute to an apoptotic response in tumor cells following acute inactivation of an oncoprotein (6).

The "Differential Signal Attenuation" Mechanism

How might this happen? Several potential regulatory schemes could contribute to the differential decay of prosurvival and proapoptotic signals on oncogene inactivation. For example, it is well documented in numerous cell culture studies that acute inactivation of various oncoproteins leads to the rapid dephosphorylation (and inactivation) of AKT, which is both a key cell survival mediator in tumor cells and a downstream target of many of the common oncoproteins (e.g., tyrosine kinases such as EGFR and Src). Although the molecular pathways that link these active kinases to an apoptotic response are poorly understood, these pathways are likely to involve a cascade of signaling events, some of which culminate in gene expression changes. Inactivation of an upstream kinase may not be sensed by downstream proapoptotic mediators for a longer period of time than that required for AKT inactivation, thereby creating a signaling imbalance that leads to an irreversible apoptotic response. Certainly, differential turnover rates of key regulatory phosphorylations could dramatically affect the activity half-life of the various signaling proteins downstream of an active oncoprotein.

Experimental findings in several systems have provided circumstantial evidence supporting this model of "differential signal attenuation." For example, following acute inactivation of BCR-ABL or Src tyrosine kinases, phospho-AKT levels are reduced within minutes whereas levels of active phospho-p38 and phospho-c-jun NH_2-terminal kinase, which have been linked to proapoptotic signaling, can be detected several hours later (7, 8). We have observed the same phenomenon with gefitinib treatment of lung cancer cells expressing an activating EGFR mutant. In a different setting, oncogene inactivation leads to rapid termination of a prosurvival signal directly tied to the activation of a proapoptotic signal. Regulation of the mitogen-activated protein kinase signaling cascade, which includes the extracellular signal–regulated kinase (ERK) 1/2, c-jun NH_2-terminal kinase, and p38 kinases that play important roles in cell growth and survival, illustrates how this could be orchestrated. Like AKT, ERK1/2 are inactivated within 30 minutes of attenuating the signal from various activated tyrosine kinases. Under normal conditions, ERK1/2 proteins indirectly keep p38, and thus apoptosis, in check by activating a phosphatase called MKP7, which dephosphorylates active p38 (9–11). Thus, acute tyrosine kinase inactivation results in rapid inactivation of ERK1/2 and a subsequent slow inactivation of the phosphatase MKP7, which in turn releases its inhibitory hold on p38 and consequently unleashes delayed but long-lasting proapoptotic signals.

Biological evidence from cell culture studies of Src-transformed cells suggests that competing proapoptotic and prosurvival mechanisms may produce the illusion of an addiction-like phenomenon. In accord with "Src addiction," fibroblasts expressing a temperature-sensitive Src kinase exhibit a transformed phenotype at the permissive temperature, and these cells rapidly undergo apoptosis upon temperature shift in reduced serum (8). Because the parental fibroblasts (without active Src) do not undergo apoptosis upon serum reduction,

this seems at first glance to represent an oncogene addiction model. However, in our own studies, we have used similar temperature-sensitive Src cells to show that if serum is present during the shift to the nonpermissive temperature, cells do not undergo apoptosis, and furthermore, if they are maintained in serum for a period of time during and after temperature shift, subsequent serum reduction also fails to induce an apoptotic response although Src has been inactivated. In this setting, serum is most likely providing a survival signal during a critical transition period; after which, cells that had seemed to be dependent on Src expression are no longer susceptible to apoptosis in the absence of this oncogenic signal. These findings clearly indicate that temperature-sensitive Src cells are not actually dependent on the activated Src kinase for survival but can seem to be addicted to Src, depending on how they are manipulated in culture. Using a small-molecule Src inhibitor to inactivate Src, we have observed the same phenomenon in cells transformed by conventional v-src. Whereas the mechanisms described above pertain to apoptotic signaling, it is conceivable that analogous mechanisms operate for differentiation and senescence pathways because, like apoptosis, the latter two phenomena are generally irreversible and have been observed following attenuation of oncoprotein signaling.

The differential signal attenuation model demands that an active oncoprotein transduces proapoptotic signals. Whereas most studies of activated oncoproteins have focused on their antiapoptotic and pro-proliferative outputs, the nature of downstream pathways that lead to programmed cell death are relatively poorly understood. However, in addition to studies describing the proapoptotic activities of the c-jun NH_2-terminal kinase and p38 kinases described above, there are a few published reports that implicate additional proapoptotic proteins as downstream targets of known oncoproteins. For example, the EGFR reportedly interacts directly with the "death ligand," FAS/CD95, to promote apoptotic actions of EGF in several settings (12). In addition, the Src kinase has been found to promote apoptosis in response to the engagement of the B-cell surface protein CD20 via activation of phospholipase-γ activity (13). Finally, the Ras oncoprotein can produce proapoptotic signals via direct interaction with a protein called Nore1 (14). Whereas these findings have begun to elucidate mechanisms of proapoptotic signaling downstream of activated oncoproteins, additional studies will certainly be required to determine which of these pathways contribute to the apoptotic cell death that is frequently seen in tumor cells following drug treatments that target the various oncoproteins.

Why does inactivation of the corresponding proto-oncogenes not lead to apoptosis in normal cells? One possibility is that the attenuation of signals emanating from an actively signaling kinase, for example, is normally a well-orchestrated process in which the apoptotic output is balanced with a sufficient antiapoptotic output. The excessive, and sometimes qualitatively altered, signaling downstream of an activated oncoprotein may result in disruption of the carefully executed signal attenuation process that occurs in normal cells. Alternatively, it is possible that the excessive apoptotic signals derived from other genetic aberrations in cancer cells shift the "balance of power" such that the slightest disruption of survival signaling following oncoprotein inactivation rapidly leads to an apoptotic outcome.

Implications of the Model

In the context of molecularly targeted cancer therapy, the concept of oncogene addiction has great appeal because it points to an "Achilles heel" for tumor cells that may render them vulnerable to the inhibitory effects of a single drug. Indeed, the clinical success of a few targeted anticancer agents has highlighted this point. It is important to note that our differential signal attenuation model similarly supports the utility of targeting oncoproteins with single agents. Moreover, it raises the possibility that drugs targeting the various downstream signaling proteins that shift the balance of proapoptotic and prosurvival signaling could also have therapeutic value. On the other hand, coadministration of drugs that induce a cell cycle arrest may suppress the apoptotic stimulus triggered by oncogene withdrawal, a possibility that is of particular interest given the disappointing results observed to date when tyrosine kinase inhibitors are administered together with classic chemotherapy drugs. It is also possible that differential signal attenuation plays some role in the acquisition of drug resistance that frequently develops in cancer patients treated with selective kinase inhibitors. Whereas secondary kinase mutations and increased activity of multidrug transporters in tumor cells account for many cases of acquired drug resistance, others remain unexplained. It is possible that genetic or epigenetic alterations render cells capable of surviving during the window of time between the decay of survival and proapoptotic signals (when apoptotic signals are proposed to be predominant), and they are thereby spared an apoptotic death.

We recognize that what we propose here is a hypothesis. Whereas several published experimental studies have produced results consistent with our model, we have presented it largely in the hope that it will prompt further experimentation to explore the proposed mechanism in a variety of systems. Most importantly, this curious property of tumor cells, whether it relates to oncogene addiction, oncogenic shock, or both, seems to have expanded the opportunities for therapeutic intervention.

Open Discussion

Dr. Tim Eisen: I was intrigued by your statement that EGFR mutations tended to occur in the younger age group and amplifications in the older age group. Could you say a bit more about that? Because I suppose the conclusion we would jump to is that these are two different etiologies at different ages. There is an alternative explanation—that things might be changing over time. Do you have any evidence of that? Also could you just say a word about mutagenicity and what you think might be causing these mutations?

Dr. Haber: As geneticists, it would be nice to think of genetic pathways linked to different types of lung cancer. We'd like to think that EGFR-mutant tumors tend to be non-smoking related; they may occur because of disruption in some biological pathway that affects a certain cell type, and they may give rise to earlier types of tumors that don't require as many additional mutations, compared with the smoking-induced lung cancers, which have typically been linked to specific mutations in p53 and RAS. There certainly are strong data linking tobacco exposure to specific genotoxic damage and characteristic mutations in p53.

With respect to amplification, we couldn't find any distinctions between the EGFR-amplified cases and the total cohort of lung cancer patients whereas EGFR mutations clearly tracked with unique histology, sex, younger age, and ethnic background. Again, we measured amplification by real-time PCR and that could be quite different from the fluorescence *in situ* hybridization criteria measured by Dr. Bunn.

Dr. Bruce Johnson: Because we have both a lung cancer and a breast geneticist present, what is the tendency in breast cancer for HER2 amplification? Does it occur in younger or older women?

Dr. Haber: I'm not aware of a major age discrepancy there, although HER2 amplification is more common in breast cancers without estrogen receptors, which is more common in older women, and that would probably lead to an age bias. I would defer to Dr. Johnson.

Dr. David Johnson: I don't think it is a huge disparity, but the mutation tends to be more common in younger women than the amplification of HER2. There are other tumor types where there are age disparities. One that is often talked about is the anaplastic oligodendrogliomas, which show the mutational abnormalities that are associated with alleged chemotherapy sensitivity. But all that occurs in younger patients, whereas lung cancer is a disease that tends to occur more in elderly. So there are differences genetically in that group of patients. As far as I know, those younger people are not predisposed to other forms of malignancy.

Dr. Bruce Johnson: With the figure you showed relating different EGFR autophosphorylation sites to distinct downstream effectors, how consistent are these correlations as you go through the different epithelial tissues that have EGFR on the cell membranes? For instance, is this consistent going from lung to breast tissue?

Dr. Settleman: We have looked in lung and breast, and what we see it is quite consistent. But we haven't looked at, say, the recruitment of all these complexes in lung and breast; we have just looked at downstream phospho-responses. So whether there is different recruitment of different complexes to different sites in different tissues, I don't know the answer to that.

Dr. Haber: I think one thing to remember is that different cells use different mechanisms for their survival signals. So here it may be that these mutant cells use EGFR to mediate most of their survival signals, but other tumor cells may use completely different pathways and have equal resistance to apoptosis.

Dr. Eisen: In relation to that, one finding that is emerging from other cell signaling systems, particularly the Ras/Raf/MEK/ERK pathway, is that although these are survival pathways, overactivation of that pathway is actually a senescence pathway. Do you have anything to say on that in this setting?

Dr. Settleman: We saw apparently reduced ERK activation in response to engaging these mutant EGFRs, but it is very hard to know whether that is happening in the tumors as well. It is in the two cell lines that we have been looking at. We also saw the same thing in another cell line model in which we introduced the mutant EGFRs into mouse mammary epithelial cells. So there does seem to be, if anything, reduced ERK output here. So I doubt we are in a scenario where we have excessive ERK signaling, and those findings are probably not relevant here.

References

1. Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heal of cancer. Science 2002;297:63–4.
2. Jonkers J, Berns A. Oncogene addiction: sometimes a temporary slavery. Cancer Cell 2004;6:535–8.
3. Weinstein IB, Begemann M, Zhou P, et al. Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy. Clin Cancer Res 1997;3:2696–702.
4. Weinstein IB. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. Carcinogenesis 2000;21:857–64.
5. Brunet CL, Gunby RH, Benson RS, Hickman JA, Watson AJ, Brady G. Commitment to cell death measured by loss of clonogenicity is separable from the appearance of apoptotic markers. Cell Death Differ 1998;5:107–15.
6. Kaelin WG, Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer 2005;5:689–98.
7. Parmar S, Katsoulidis E, Verma A, et al. Role of the p38 mitogen-activated protein kinase pathway in the generation of the effects of imatinib mesylate (STI571) in BCR-ABL-expressing cells. J Biol Chem 2004;279:25345–52.
8. Johnson D, Agochiya M, Samejima K, Earnshaw W, Frame M, Wyke J. Regulation of both apoptosis and cell survival by the v-Src oncoprotein. Cell Death Differ 2000;7:685–96.
9. Masuda K, Shima H, Katagiri C, Kikuchi K. Activation of ERK induces phosphorylation of MAPK phosphatase-7, a JNK specific phosphatase, at Ser-446. J Biol Chem 2003;278:32448–56.
10. Tanoue T, Yamamoto T, Maeda R, Nishida E. A Novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 α and β MAPKs. J Biol Chem 2001;276:26629–39.
11. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326–31.
12. Reinehr R, Schliess F, Haussinger D. Hyperosmolarity and CD95L trigger CD95/EGF receptor association and tyrosine phosphorylation of CD95 as prerequisites for CD95 membrane trafficking and DISC formation. FASEB J 2003;17:731–3.
13. Hofmeister JK, Cooney D, Coggeshall KM. Clustered CD20 induced apoptosis: src-family kinase, the proximal regulator of tyrosine phosphorylation, calcium influx, and caspase 3-dependent apoptosis. Blood Cells Mol Dis 2000;26:133–43.
14. Vos MD, Martinez A, Ellis CA, Vallecorsa T, Clark GJ. The pro-apoptotic Ras effector Nore1 may serve as a Ras-regulated tumor suppressor in the lung. J Biol Chem 2003;278:21938–43.

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