

Is Co-option a Prevailing Mechanism during Cancer Progression?

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Abstract

Cancer progression results from the accumulation of genetic and epigenetic alterations that provide tumor cells with a selective advantage. The consecutive cycles of mutations, selections, and clonal expansions generate, over time, descendant cells with increasing malignant properties. Although this conception of tumor development rests on solid experimental foundations, it has also raised several persisting questions. Does the succession of mutations dictate the progression of each cancer type or does the disturbance of an invariant set of regulatory circuits govern tumor evolution regardless of the linear order of genetic events? Is the ability of malignant cells to disseminate and spawn metastasis a property acquired at late stages of tumor development or is the proclivity to metastasize implanted early during cancer formation? Considering these issues, we elaborate here on the concept of co-option that refers to the emergence of novel functions from ancestral characters during episodes of organismal evolution. As discussed in this Perspective, co-option seems to be a key mechanism propelling the molecular engine that drives malignant transformation. Hence, this notion may constitute a unifying principle that connects a large body of experimental results to clinical observations. *Cancer Res*; 71(21); 6572–5. ©2011 AACR.

Setting the Problems

Cancer progression is conventionally viewed as a process that recapitulates Darwinian evolution (1). According to this model, the emergence of malignant cells results from the sequential selection of a clonal progeny that has acquired the capacity to escape physiologic fail-safe mechanisms and gradually overruns adjacent normal cells (1). Considerable evidence supports the model describing the tumor outcome as the consequence of a succession of mutational events that fosters the appearance of cells endowed with increasing neoplastic properties, such as unlimited growth, resistance to apoptosis, and induction of sustained angiogenesis (2). To date, 350 of the 22,000 human genes have been recurrently found mutated in human neoplasms, and the identification of alterations that corrupt the functions of proto-oncogenes and tumor suppressors have made this theory of carcinogenesis a forceful paradigm (3). Despite this remarkable achievement, several important questions are still awaiting completely satisfactory answers. One of them refers to the correspondence between specific genetic changes and cancer progression. It has been argued that both the summation of mutations disrupting the function of key genes but also the ordered succession of these events govern tumor evolution (4). However, evidence support-

ing the existence of a linear order of genetic paths has been scant for the large majority of malignant tumors. The second question relates to the metastatic process. The ability to invade and disseminate in the organism has been initially construed as a final step during oncogenesis that concerns rare neoplastic cell clones arising within the primary tumor and carrying mutated alleles that promote metastatic outgrowth (5). This notion has been disputed on the basis of theoretical considerations and experimental grounds (6). It has been alternatively proposed that a subset of mutated alleles conferring a selective advantage to cells forming the primary tumor are the very same alleles that trigger at later stages the capacity to colonize distant organs (6). Taking into account these enduring questions, we examine here how co-option and related concepts elaborated in the field of evolutionary biology may provide insights into these issues.

Co-option and spandrels in the theory of evolution

The biologist George Mivart formulated consistent objections after the publication of the "Origin of Species," pointing out that if evolution proceeds through a series of small gradual changes, then the selective advantage of novel traits or structures at incipient stages is equivocal. In his answer, Charles Darwin reemphasized that a biological structure might shift of function during the evolutionary history of an organism and acquisition of this novel function might be required in a different environment (7). This phenomenon is referred to as co-option or recruitment. For example, feathers were probably initially selected as an efficient thermoregulator for the ancestors of birds and later during evolution became adapted to flying. Although the molecular bases of co-option are not precisely known, clear examples of this process have made it possible to distinguish two broad classes: structural gene

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co-option and developmental pathway co-option (8). The former mode involves either modification in the amino acid sequence of proteins that is associated with novel functions or gene duplications and adoption of novel functions by the daughter paralogs (8). The latter corresponds to changes in gene regulation that leads to spatiotemporal variations in the pattern of gene expression. This reiterated use of genes in different contexts contributes to the edification of novel morphogenetic structures (8). If co-option designates a biological function whose current adaptive values were not the function conducted when it was originally selected, some presently beneficial functions may originate from features that were not initially adaptations. Gould and Lewontin called these non-adaptive by-products that "become available for later and secondary utility" (9) "spandrels". In masonry vocabulary, spandrels are triangular spaces between two arches or between an arch and a supporting dome. These structures result from architectural constraints and were not designed for a specific function but were subsequently ornamented by painters for decorative purposes. According to the metaphor by Gould and Lewontin, some biological features may similarly arise as inevitable by-products and may then be co-opted for novel adaptive functions (9).

Gain of function is co-option

What may be then the value of these concepts in cancerology? Genetic damages of proto-oncogenes, either point mutations or chromosomal rearrangements, lead to the formation of oncoproteins whose biological functions usually differ from their normal counterparts. This phenomenon referred as a "gain of functions" has been largely documented for tyrosine kinases (10, 11, 12). Molecular alterations of *ABL*, *EGFR*, *RET*, *KIT*, *MET*, and *FLT3*, to name a few, produce kinases that not only display a constitutive enzymatic activity but also: (i) alter their substrate specificity; (ii) give access to novel substrates through modified subcellular localization of the mutant kinases; and (iii) perturb the duration of the intracellular signal transduced, thereby triggering the stimulation of pathways or biological programs unrelated to their primary signaling network (10–12). Another compelling example is the recent demonstration that missense mutation at arginine 132 of the isocitrate dehydrogenase 1 (*IDH1*), a genetic change frequently found in gliomas, generates an enzyme that produces the 2-hydroxyglutarate oncometabolite instead of solely converting isocitrate in α -ketoglutarate (13). Thus, reformulated in the evolutionary developmental terminology, these cases are clear proofs that structural gene co-option occurs during tumor progression. In addition, examples of genes mostly expressed during organismal development and aberrantly redeployed in tumors cells are abundant in the oncology corpus. A recent illustration is the demonstration that the gene encoding the embryonic M2 isoform of pyruvate kinase (*PK-M2*) is turned on in tumor cells, thereby contributing to the reprogramming of energetic metabolism that specifies cancer cells (14). Altogether, these findings persuasively indicate that functional shifts known to characterize co-option are prevalent mechanisms involved in the evolution of malignancy.

Co-option in cancer metastasis

Do we have any molecular evidence for the involvement of co-optive mechanisms during the formation of metastasis? Before discussing this issue, it is opportune to come back to what is precisely meant by developmental pathway co-option. According to the classification made by True and Carroll (8), this situation supposes that "ancestral gene functions such as transcriptional regulation and components of cellular signaling pathways have been conserved, but the genes have been redeployed in different tissues and developmental stages during evolution."

If we consider this definition, several cases of developmental pathway co-option during the metastatic process have been described over the past decade. One of the best examples concerns the epithelial-mesenchymal transition (EMT), a reversible physiologic process that is critical during several phases of the embryonic development such as gastrulation and formation of the neural crest (15). Epithelial cells that undergo EMT lose expression of molecules contributing to the epithelial phenotype such as the intercellular adherence molecule E-cadherin and acquire fibroblastoid motile properties (15). It is becoming widely accepted that inappropriate recapitulation of this developmental program, or alternatively stated its co-option by tumor cells, contributes to the metastatic dissemination to distant organs (15). Furthermore, epithelial cells that undergo EMT adopt stem cell properties that confer self-renewable properties to metastatic malignant seeding cells (16). *TWIST*, *Slug*, and *Snail* are key transcription factors that drive EMT by repressing transcription of the E-cadherin locus (15). Ectopic expression of these proteins is known to promote invasiveness of epithelial tumor cells. Interestingly, *TWIST* counteracts oncogene-induced senescence in incipient tumors and fosters the spreading of malignant cells via its EMT-inducing activities, thus raising the idea that its unscheduled expression might contribute to the early dissemination of neoplastic cells (17).

Another example that does not match exactly the definition of a developmental program co-option corresponds to the metabolic rewiring known as Warburg effect (18, 19). This process refers to the fact that under aerobic conditions, cancer cells metabolize glucose and produce ATP mostly through glycolysis. Several reasons have been invoked to explain the Warburg effect such as an adaptation of nascent tumor cells to the fluctuations of oxygen level in their initial microenvironment and the need to face enhanced biosynthetic demands for cell proliferation via the funneling of the glycolytic pathway intermediates towards macromolecular synthesis (18, 19). A number of oncoproteins and tumor suppressors whose activities are derailed in tumor cells participate in this metabolic shift. However, several experimental arguments plead also for a role of the enhancement of glycolytic flux during metastasis. Acidification of the extracellular microenvironment via the export of lactic acid and proton-extruding transporters contributes to the invasive behavior of tumor cells and interferes with the activity of natural killer cells (20, 21). Furthermore, stabilization of the hypoxia-inducible factor 1 alpha (*HIF-1 α*) in response to hypoxic conditions, ectopic expression of *PK-M2*, shortage of nutrients, or via oncogenic activation is essential to trigger EMT (22, 23, 24). Thus,

Warburg effect can be considered as a metabolic adaptation to the initial conditions of tumor development that is co-opted for the metastatic process. Moreover, decrease of the extracellular microenvironment pH is an intrinsic by-product of aerobic glycolysis, or alternatively stated, a biochemical spandrel, that favors tumor cells dispersion and is co-opted to escape attacks of the immune system.

A link between co-option and metastasis has also been identified during the process that mediates invasion of breast cancer cells to the lungs (25). The products of four genes, epiregulin, COX2, and matrix metalloproteinases 1 and 2, support the assembly of new vasculature and favor tumor extravasation from pulmonary capillaries. Consistently, extinction of their expression elicits apoptosis in the primary tumor, thus proving that these genes elicit growth advantage to the tumor cells *in situ* and are co-opted during metastatic spreading (25). Another situation that has been extensively studied describes the capacity developed by solid tumor cells, especially in the brain, to use preexistent host vasculature to obtain blood supply and to colonize distant sites (26). This acquired property of malignant cells is recognized as "vessel co-option" (26).

A last series of examples illustrates the relation between hemostasis and tumor spreading. It is well known that a prothrombic state is associated with the development of different types of solid tumors (27). Hemostasis activation by cancer cells leads to fibrin deposition at the tumor site that provides a provisional extracellular scaffold permissive for neoangiogenesis and cell dissemination (27). A recent study has shown that expression of the *MET* oncogene in hepatocytes led to liver cancer and was accompanied by a disseminated intravascular coagulation phenotype (28). The authors found that permanent and systemic expression of both the plasminogen activator inhibitor type 1 (PAI-1) and COX-2 induced by *MET* was responsible for venous thromboses formation. Thus, expression of the *MET* oncoprotein promotes the formation of primary liver neoplasms and dysregulates a hemostasis genetic program that is co-opted for tumor cell dissemination. A further link between blood coagulation disorders and tumor spreading refers to the observation that cancer cells are frequently found within microthrombi (29). The platelet-aggregating activity of tumor cells is believed to facilitate their traveling into blood vessels by ensuring protection from the hydrodynamic shear forces and from destruction by immune competent cells (29). The coating of malignant cells with platelets favors also their extravasation by facilitating adhesion to the endothelium and induces neoangiogenesis through the release of various cytokines including the VEGF. A recent study provided evidence that lysophosphatidic acid (LPA) is produced by platelets in the tumor microenvironment and exerts a mitogenic effect on breast and ovarian cancer cells (30). Furthermore, LPA enhances the formation of osteolytic bone metastasis by stimulating tumor cells to secrete soluble factors that potentiate osteoclast activation (30). Thus, as already pointed out (31), LPA production is co-opted by malignant cells, as it enhances tumor cell proliferation at early stages of circulating dissemination and fosters bone metastasis at later stages.

Together, these examples persuasively indicate that functional shifts typifying co-option occur at several steps of the

metastatic cascade. A recent model of invasion asserts that a set of mutated alleles that is selected during cell transformation and promotes tumor outgrowth is the same set that confers to malignant cells their invasive behavior and capacity to thrive in novel microenvironments (6). As reviewed here and according to the logic of this scenario, there is empirical evidence that a fraction of genes and developmental pathways causing primary tumor development are indeed co-opted during the metastatic process.

Addiction and co-option

To complete this Perspective, we would like to emphasize possible implications of the notions discussed earlier. First, the existence of co-optive mechanisms questions the model of tumor progression based on the temporal order of genetic alterations (4). If a very same event could be translated into distinct biological outputs at different stages of the neoplastic evolutionary process, it may become deceptive to try to correspond genetic alterations with their presumed biological effects at a single step of the tumor progression. As insightfully proposed by Bernards and Weinberg (6) and commented here, the same mutated gene can contribute to tumor initiation and metastasis formation. Thus, the evidence of co-optive processes challenges models that strictly ascribe a causative role of genetic modifications to a given clinical stage of tumorigenesis.

The second point refers to the "oncogenic addiction," a phenomenon describing the strict dependency of malignant cells on the sustained activity of an oncogene expressed in the very same cells (32). This is seemingly trivial, but is in fact puzzling, because it indicates that cancer cells, despite the acquisition of multiple genetic abnormalities, are "hooked" to the function of a single transforming gene, even when the oncogenic mutation is an early event in the tumor process. Several consistent explanations, not mutually exclusive, and accounting for the addictive neoplastic features have been put forward (33). However, it is also relevant to posit that molecules participating in the initial conditions of tumor formation, and co-opted for additional functions, render malignant cells dependent on this early molecular event during the final phases of the pathologic process. Therefore, in certain circumstances, oncogene addiction and oncogene co-option may well be the two faces of the same coin.

Conclusion

The views discussed here enforce the idea initially proposed by Darwin, relayed by Gould and other biologists, that caution should be exerted when attempting to infer current utility of a trait from the causes of its historical origin. This lesson should be kept in mind when assigning defined roles to early tumorigenic events and extrapolating their eventual contribution to later steps from their initial causative functions. The high degree of connectivity between signaling pathways and the pleiotropy of proteins involved in these networks offers multiple opportunities for co-option during the time scale of tumor evolution. In addition, the notion of spandrels draws attention to a fact which may be the source of confounding interpretations, that is, invariant features of malignant cells may not be

traits that have a selective edge but may result from biochemical constraints that are "hardwired" in signaling and metabolic pathways.

Several recent reports have revealed unexpected mechanisms that do not fit the gradualist scenario involving the stepwise genetic modifications of cells toward malignancy but resemble the "punctuated-equilibrium" model of speciation (34–36). In this review, we have proposed that co-option, another concept initially developed to resolve questions raised by the Darwinian theory, may also have broad applicability in oncology. Finally, from a clinical standpoint, it is worth underscoring that components of the genetic regulatory networks co-opted during tumor progression will constitute, when identified, prime choices for designing targeted drugs.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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