Re: A Model of Human Tumor Dormancy: An Angiogenic Escape From the Nonangiogenic Phenotype

We have read with great interest the study published by Naumov et al. (1), which provided a novel model of human tumor dormancy. However, we have some concerns regarding their basic assumptions and conclusions.

First, the basic assumption in the study by Naumov et al. is that tumor growth beyond the size of 1–2 mm is completely dependent on the angiogenic switch. However, the hypothesis that solid tumor growth is completely dependent on angiogenesis (angiogenic switch), which has prevailed for many years, has recently been challenged with the observations of angiogenesis-independent growth (referred to in the literature as nonangiogenic tumor growth). Solid tumor growth beyond the diffusion-limited size of 1–2 mm without elicited angiogenesis and with co-option of native vasculature of host tissue has been reported with increasing frequency and has been described in non–small-cell lung cancer (NSCLC), lung metastases, liver metastases, skin metastases, and lymph node metastases (2–4). Nonangiogenic tumor growth is not an exception; its frequency varies from 16% to 96% depending on the organ and the primary tumor (2–4).

An essential prerequisite for nonangiogenic tumor growth appears to be the ability of the tumor to preserve the stromal architecture of the host tissue as well as the pre-existing blood vessels. Indeed, in NSCLC, an alveolar nonangiogenic tumor growth has been described in which tumor cell nests fill the alveolar spaces without destruction of the lung parenchyma, co-opting the septal blood vessels (2). Subsequent studies also suggested that the alveolar growth pattern is nonangiogenic in that incorporated blood vessels had the same phenotypic characteristics of native alveolar blood vessels, and a three-dimensional reconstruction of the tumor confirmed the preservation of stromal architecture of the lung (5,6). We have shown that an alveolar growth pattern is associated with poor prognosis in NSCLC and that this growth pattern is indeed nonangiogenic, being characterized by a low endothelial cell proliferation fraction and a high tumor cell proliferation fraction (7).

These observations add weight to the contention that tumor growth requires adequate vascularization rather than always being dependent on angiogenesis. We therefore argue that nonangiogenic phenotype is a specific disease entity rather than a pre-angiogenic state.

Second, an important question regarding the study of Naumov et al. is whether the observed angiogenic switch in a nonangiogenic tumor cell line was independent of the methodology. The nonangiogenic cell lines used in the study were probably completely dependent on angiogenesis because these cell lines could not grow in vivo beyond 1 mm in diameter. Although these cell lines were angiogenesis dependent, they probably, at inoculation, lacked the ability to induce angiogenesis. Naumov et al. have shown that these nonangiogenic cell lines, even before they switched from dormancy to the angiogenic phenotype, had high proliferation rates and were therefore not quiescent. It is also clear from their results that an angiogenic switch occurred in only a minority of mice inoculated with nonangiogenic cell lines, and only after at least 130–238 days. Therefore, it is possible that a nonangiogenic cell line with a high proliferation fraction could, after a long observation period, create subclones with the angiogenic switch through subsequent mutations. If so, then the cell line before and after the angiogenic switch would not be genetically identical. This has to be clarified before this model can be used for further studies.

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Response
We appreciate the comments of Sardari Nia et al. It is well established in the literature that all expanding tumors need a supporting vasculature. Sardari Nia et al. contend that large tumors can grow by co-option, a process that was first described by Holash et al. (1), who demonstrated that tumor cells can grow as perivascular cuffs in thickness no more than the oxygen diffusion limit. The oxygen diffusion limit has been defined previously as 200–250 µm or less (2,3). Beyond this tumor thickness, virtually all published studies reveal the requirement for neovascularization, i.e., new vessel sprouts, a hallmark of the angiogenic process (4). In contrast, the co-option

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process has not been shown to be dependent on vascular sprouting or to be capable of supporting tumor growth beyond 1–2 mm in diameter.

In some instances, tumor growth by co-option may follow the stromal architecture of the host tissue, as exemplified by Sardari Nia et al. Such tumors would be appropriately called “nonangiogenic” because no new vascular sprouting was observed. It appears that the authors’ main criticism of our study is with our use of the term “nonangiogenic” to describe one form of the dormant tumor phenotype. This use of the term apparently conflicts with their use of the term, to describe tumors that do not appear to require angiogenesis for continued increase in mass. However, we stated clearly at the outset of our article that blocked angiogenesis (i.e., absence of angiogenesis) is not the only mechanism of tumor dormancy. For example, hormone deprivation or immune responses can also induce tumor dormancy. We are puzzled about how the semantic argument by Sardari Nia et al. bears on the results we describe or the conclusions we draw from them.

It is possible that co-option of the tumor vascular supply may play a role in the maintenance of microscopic tumors (1), but we know of no strong evidence that macroscopic tumors with increasing mass can grow by co-option alone. The proof of co-option as a method of permitting large mass of tumor growth without new vascular sprouts would require careful confocal microscopy coupled with the use of an intravascular dye, such as lectin (5). Turner et al. (6) emphasized that tumor angiogenesis can also be measured in vivo by magnetic resonance imaging using a contrast agent targeted to the α,β integrin, which is more strongly expressed on angiogenic than on normal blood vessels. Alternatively, using histologic methods, it is practical to use an antibody (e.g., LH39) that recognizes an epitope found only in mature vessels and to compare the presence of this epitope with vascular counts using CD31, which recognizes both mature and newly formed vessels. This latter approach allows a measurement of active vascular remodeling, rather than a static vessel count (6).

Whereas angiogenesis, by definition, requires new microvascular sprouts (4, 5), these sprouts can grow by endothelial migration even in the absence of endothelial cell proliferation (7). Therefore, we do not feel that co-option and angiogenesis are mutually exclusive. They may, in fact, coexist in the same tumor.

Sardari Nia et al. also suggest that the switch to an angiogenic phenotype by the tumor cells we used could be mediated by genetic mutations occurring during the period (months) of nonangiogenic existence in vivo. In our study, we did not assert that tumor cells emerging from an angiogenic switch are genetically identical to those that existed before the switch. On the contrary, multiple genetic and epigenetic events are likely to occur during the acquisition of an angiogenic phenotype as tumor growth proceeds. For example, in our paper, we demonstrated an increased expression of the Myc oncogene in angiogenic tumor cells compared to nonangiogenic tumor cells.

In summary, it is not uncommon in a rapidly developing new field, such as angiogenesis research, for conflicting interpretations to arise and for disagreements to emerge because discoveries can outpace methodology.

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References