Comments to the Editor

Reply to Comments by Buceta and Galeano Regarding the Article ''The Universal Dynamics of Tumor Growth''

In their comment on the article ''The Universal Dynamics of Tumor Growth'' by Brú et al. (2003), J. Buceta and J. Galeano imply that the analysis presented is incorrect and lacking in rigor. The article in question shows, using scaling analysis, that 16 in vitro-grown tumor lines and 15 in vivo tumors all have the same growth dynamics. These results support the conclusions made in a previous article by our group (Brú et al., 1998), in which the methods for analyzing circular interfaces were developed. In that article, these methods were used to determine the growth dynamics of four clones of the C6 astrocytoma cell line. The critical exponents describing the spatial and temporal invariances of the interface were determined and found to be completely compatible with the molecular beam epitaxy (MBE) universality class. For later discussion, it is important to note that the contours of the clones clearly grew over the 1400 h cultivation period but that this did not alter the results of the scaling analysis—thus the growth dynamics of the tumors did not change either. The values for the critical exponents of local and global roughness were verified using several methods developed (and later published) for use with interfaces that change in size over time (Brú and Casero, 2003). This latter article compares three methods for measuring the local width of an interface and shows that the local roughness exponent values do not change simply because the system changes in size. In fact, this article analyzes models that show the apparent scaling anomaly to be an effect of the interface analysis methodology. In Brú et al. (2003) (the article commented upon by Buceta and Galeano), we show how the growth dynamics of 15 tumor lines and nontumor cell colonies, and 16 animal and human tumors in vivo, are all completely compatible with MBE universality—strongly suggesting that these dynamics describe the growth of all tumors. Moreover, the spectra provide a coefficient of global roughness of 1.5; the only universality class compatible with this value is MBE (as clearly shown in the figures in the article), as well as the rest of measured critical exponents.

In this article (Brú et al., 2003), we clearly state that if all tumors grow with MBE dynamics, then they should also show three classic features of the MBE system. The first of these is a linear growth rate (Brú et al., 1998), something easily shown by averaging the terms of the standard MBE universality equation. The second, which in some ways is an implication of the first, is that any growth will be restricted mainly to the border of the system (Brú et al., 1998). Finally, the third is arrived at by considering the fourth derivative of the MBE universality class equation (which expresses the dominant mechanism of the growth process), and this clearly shows that tumor growth occurs by diffusion of newly produced cells at the tumor border. These three characteristics can all be deduced from the MBE equation, which we believe represents the growth dynamics of all solid tumors.

It might be said that the growth dynamics of a system are ''written in its interface''. Scaling analysis is one of the most powerful tools ever developed for determining the dynamic component of systems with rough interfaces. Analysis of the spatial and temporal invariances allows the growth dynamics to be determined—and therefore the mechanisms responsible for growth. These invariances are quantified using the values for a set of very robust critical exponents. Scaling analysis thus allows a standard growth equation to be determined, and with this the universality class of the dynamics and the basic growth mechanisms of the system. It is this that most surprises people not familiar with scaling analysis but which so obviously marks the difference with what might be called conventional modeling. The latter tries first to determine the hypothetical mechanism of growth and from this the mathematical equation that governs the process. Scaling analysis allows the reverse.

The argument put forward by Buceta and Galeano is, therefore, just the opposite of a scaling analysis approach. They first argue that a system with a constant growth rate does not necessarily mean that MBE dynamics are at work. What they fail to realize is that implications do not always work in both directions. It is absolutely clear that not every system with a linear growth rate has to have MBE dynamics, *but every system with MBE dynamics has to have a linear growth rate* (this can be obtained theoretically by averaging the terms in the equation).

Secondly, Buceta and Galeano criticize the implication that MBE dynamics must mean that growth is restricted to the system's border. But this is precisely what all systems with MBE dynamics—and we know of many—*always show*. The basic mechanism of growth is most certainly diffusion of cells at the tumor border, as the values of the critical exponents reveal. Not only are there a great many biological reasons why this should be so (including the fact that the cells inside a tumor have no room to grow), we show this to be true experimentally in 16 cell lines and 15 tumors. Not only is this theoretically and experimentally the case, it is the main point we make in our article. If the cells at the border are the only ones that proliferate, then whatever is inhibiting those in the center from growing could provide the basis of

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Address reprint requests to Antonio Brú, E-mail: antonio.bru@ccma.csic.es.

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an antitumor therapy (Brú et al., 2004; A. Brú, S. Albertos, F. Garcia-Oz, and I. Brú, unpublished).

Also, Buceta and Galeano state that several different types of diffusion process exist. This is true, but it is also thoroughly well known that not all are a product of MBE dynamics. What we state in our article is that if tumors grow with MBE dynamics, *then there must be a diffusion process*; and we provide the experimental evidence that confirms it.

Buceta and Galeano take the characteristics mentioned above *independently* and argue that on their own they do not imply MBE dynamics are at work; this is their premise for believing our work is not sufficiently rigorous and that it might even be incorrect. But we never once say that they do imply MBE dynamics are present, and much less independently. What we say is that the system *has MBE dynamics and therefore shows these characteristics*. Indeed, a question our critics do not answer is whether these three experimentally confirmed characteristics acting together could be a reflection of anything else.

Finally, and equally unjustifiably, Galeano and Buceta argue that the incoherence and the error of our analysis stem from not taking into account the spatial dilation they describe (Galeano et al., 2003). We here remind the reader that our work was experimentally confirmed using the method of Brú and Casero developed for measuring the local roughness of systems with interfaces that change in size (Brú and Casero, 2003). In this earlier article, we argued the well-known fact that in systems in which the interface varies in size, the interface width need not saturate (as Buceta and Galeano quite rightly state). However, the results they present on the growth of calluses cannot be extrapolated to tumor growth. The interfaces of these calluses did not change in size over time, whereas those of our cell colonies did (by several orders of magnitude over the experimental period; if there were any effect influencing scaling, we would certainly have seen it, especially since the length of time over which we cultured our cell lines is rather longer than anything that can be found in the literature). The lack of growth of the calluses in Bruceta and Galeano's work is clearly reflected in the fact that their interface spectra (which were very noisy) showed no temporal changes. This explains the collapse they obtained; the spectra did not change over time, so a collapse was inevitable from the outset with the spatial dilation factor they propose (in fact the results would have been the same for many other spatial dilation factors). Thus, they specify no growth rate because there is none to give (the contours at different times they provide all fall within the spatial discrimination). In addition, all the calluses developed similarly (almost within the spatial discrimination) and curiously with the same fractal dimension (which appears not to change over time). These authors even make the claim that the same fractal dimension should occur in all plant species. Nevertheless, the analysis presented in that article has nothing to do with tumor growth, not because of the methodology, which is the same as that used in (Brú et al., 1998), but because of the nature of the system studied and the biological interpretations that can be made (in fact Galeano and Buceta fail to make any biological conclusions). Indeed, the dilation proposed by these authors has never been experimentally validated in any other known dynamic system.

In finishing, we would like to point out that the characteristics of MBE dynamics discussed in Brú et al. (2003, 1998) have not only been rigorously demonstrated but have served as the basis for a successful antitumor therapy currently under development (Brú et al., 2004; A. Brú, S. Albertos, F. Garcia-Oz, and I. Brú, unpublished).

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Antonio Brú,*† Sonia Albertos,‡ José Luis Subiza,§ José Lopez Garcia-Asenjo,¶ and Isabel Brú‖

*Dpto. Matematica Aplicada
Facultad de CC. Matemáticas
28040 Madrid, Spain

†CCMA
Consejo Superior de Investigaciones Científicas (CSIC)
28006 Madrid, Spain

‡Servicio de Aparato Digestivo
Hospital Clínico San Carlos
28003 Madrid, Spain

§Servicio de Inmunología
Hospital Clínico San Carlos
28003 Madrid, Spain

¶Servicio de Anatomía Patológica
Hospital Clínico San Carlos
28003 Madrid, Spain

‖Centro de Salud La Estación
Paseo del muelle 70
45600 Talavera de la Reina,
Toledo, Spain