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Letter to the Editor

Response to the letter of B. Carvalho "Chromosomal instability, aneuploidy, and gene mutations in human sporadic colorectal adenomas" published in Cellular Oncology Vol. 27(4), 2005, p. 267

To the Editor:

The letter of Dr. B. Carvalho raised different interesting points of discussion on the data presented in our recent review article in Cellular Oncology [13].

Among human sporadic colorectal adenomas without early cancer, we found a statistically significant association of DNA aneuploidy, as assessed by flow cytometry, with *KRAS2* G > C and G > T transvertions but not G > A transitions [14], with APC mutations within and downstream the mutation cluster region but not upstream [15], and with deletions at 1p [10].

First of all, it must be clearly said that the mechanisms of Chromosomal Instability (CIN) and aneuploidy (a bona fide consequence of CIN) are still poorly understood. Nevertheless, CIN and aneuploidy are thought to be key genomic events in the colorectal adenoma–carcinoma sequence and in the genesis and progression of other tumor types [5,11,18,20,27–32, 37–39].

We were presently invited to speculate on the role of RAS in CIN, including the possible mechanisms involved. This controversial issue was very recently reviewed and the *in vitro* and *in vivo* findings that led several authors to suggest the possible role of *RAS* mutations in CIN were discussed [6].

Using *Schizosaccharomyces pombe*, it was proven that the interaction of RAS-dependent specific proteins affects the cytoskeleton and the mitotic spindle [7,21,36] and might therefore affect CIN and aneuploidy.

Using rat thyroid follicular cells, it was also suggested that *RAS* mutations may predispose to aneuploidy by affecting centrosome amplification and chromosome misalignment [34].

Similarly, using *in vitro* rodent cells, the increased constitutive ERK activity resulting from *RAS* or *MOS* oncogene expression was shown to lead to altered microtubule dynamics, spindle disturbances, mitotic aberrations and generation of whole chromosome-containing micronuclei [12,35].

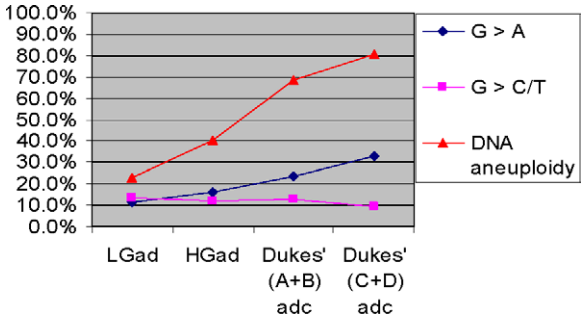
Enhanced karyotipic instability [8], increased rate of abnormal mitoses [17,33], aneuploidy as detected by flow cytometry [25], inhibition of apoptosis and reduction of the duration of the G2M cell cycle phase [26] were also observed in 3T3 murine fibroblasts transfected with *RAS* mutated oncogenes suggesting that CIN is indirectly linked with increased survival and decreased apoptosis.

Similarly, using rat enterocytes, experimental evidence was provided that increased resistance to induced apoptosis [3] and increased expression of Ccdn1 and of the Rb1 tumor suppressor gene [4] were dependent on *KRAS* mutations.

RAS mutations were also reported to alter biochemical pathways toward the production of reactive oxygen species [19,22], which may potentially lead to DNA damage and genomic instability [9].

Dr. Carvalho also invited us to comment on the biological consequences that different *KRAS2* mutations may lead to. The type of *KRAS2* mutation was shown to affect differently the structural conformation of the protein, particularly when it is in the active GTP-bound state, and in turn to affect differently the GTPase activity and affinity for GTPase activating proteins and the interaction of the mutated KRAS2 protein with binding proteins or down-stream effectors [1,2,24].

Another interesting observation raised by Dr. Carvalho was that *KRAS2* G > A transitions were found to be more frequent than G > C and G > T transvertions in colorectal progressed adenomas [18]. We can confirm this observation with a greater number of cases. The figure shows that while *KRAS2* G > C/T transvertions are at almost constant level (between 10 and 15%) during the adenoma–carcinoma sequence, the G > A transitions frequency raised from about 10% to above 30%. DNA aneuploidy incidence is shown to be between 20 and 40% for adenomas and up to about 80% for carcinomas. (The data points in figure correspond to the following groups: LGad, adenomas with low grade dysplasia ($N = 88$); HGad, adenomas with severe dysplasia and adenomas containing or proximal to cancer ($N = 101$); (A + B)adc, adenocarcinomas



with Dukes' A and B ($N = 266$); (C + D)adc, adenocarcinomas with Dukes' C and D ($N = 186$).)

It is not simple to comment on these data. In general, it is conceivable that specific gene mutations and numerical–structural chromosomal aberrations occurring during the colorectal adenoma–carcinoma sequence cooperate to the selection among different pathways. The TP53 mutations, in particular, were found to occur as late events at the transition from adenoma to carcinoma and were also linked with CIN and aneuploidy [23]. The present data suggest that *KRAS2* G > A activation may be a late event in a subgroup of DNA aneuploid adenocarcinomas as earlier observed by investigating the intratumor heterogeneity of KRAS2 mutations in colorectal adenocarcinomas [16].

We may conclude by saying that there are many different pathways to reach the top of a hill. We certainly agree with Dr. Carvalho that the role of CIN and aneuploidy in the context of colorectal carcinogenesis and, in particular the role of *KRAS2* in CIN and aneuploidy, still hold many unknown facets and enigmas.

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