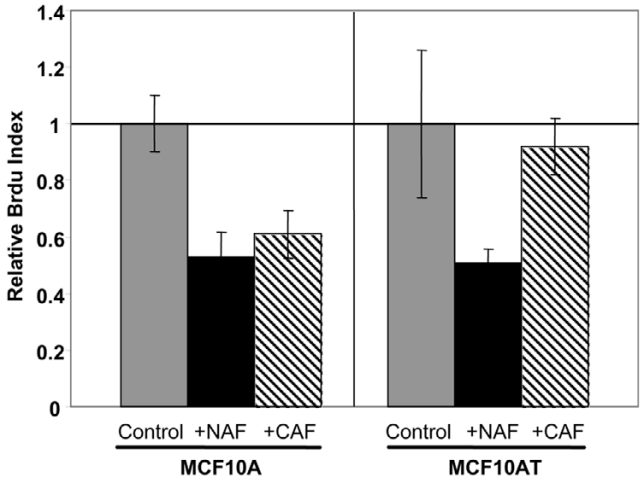
Table 1

5-Bromo-2'-deoxyuridine (BrdU) labeling* of MCF10A cells grown in monoculture (control group) and in co-cultures with normal breast-associated fibroblasts (NAF) and carcinoma-associated fibroblasts (CAF)

Culture	BrdU-labeling indices of MCF10A cells (mean ± standard error of the mean)	BrdU-labeling indices of MCF10A cells (group mean ± standard error of the mean)	Comparison of BrdU-labeling indices of MCF10A cells between groups (linear regression)	
MCF10A ($n = 6$)	30.3 ± 3.0	30.3 ± 3.0	MCF10A vs MCF10A + NAF ($P = 0.009$)	MCF10A + NAF vs MCF10A + CAF ($P = 0.501$)
NAF-1 + MCF10A ($n = 7$)	21.9 ± 4.2	16.1 ± 2.6 ($n = 19$)		
NAF-2 + MCF10A ($n = 6$)	10.7 ± 3.6			
NAF-3 + MCF10A ($n = 6$)	14.7 ± 5.0			
CAF-1 + MCF10A ($n = 8$)	15.2 ± 2.0	18.5 ± 2.5 ($n = 19$)	MCF10A vs MCF10A + CAF ($P = 0.024$)	
CAF-2 + MCF10A ($n = 6$)	15.5 ± 4.2			
CAF-3 + MCF10A ($n = 5$)	27.6 ± 6.7			

*Assessed by immunocytochemistry

Figure 4



Proliferation of MCF10A cells and MCF10AT cells grown in monoculture and co-culture with fibroblasts. The rate of proliferation of MCF10A cells and MCF10AT cells, as measured by the 5-bromo-2'-deoxyuridine (BrdU) labeling index (assessed by immunocytochemistry), was significantly reduced in co-cultures of MCF10A cells with both normal breast-associated fibroblasts (NAF) ($P = 0.009$) and carcinoma-associated fibroblasts (CAF) ($P = 0.024$) compared with the MCF10A monoculture (control). The rate of proliferation of MCF10AT cells was significantly suppressed by NAF ($P = 0.013$) but not by CAF ($P = 0.935$) in comparison with the MCF10AT monoculture (control).

index ($n = 22$, $P = 0.935$) (Table 2 and Fig. 4). The effect of NAF versus CAF on the rate of proliferation of MCF10AT cells was significantly different ($P < 0.001$). The effect was further confirmed by repeating the co-cultures to measure the BrdU-labeling index by flow cytometry, rather than by immunocytochemistry (Fig. 5).

There was variability among NAF cultures and among CAF cultures in their ability to suppress proliferation of MCF10A cells and MCF10AT cells (Tables 1 and 2) in this 3D culture system, potentially reflecting heterogeneity among the individuals from which the fibroblasts were derived. Because of this variability, detection of a significant difference in the function of NAF and CAF required many replicates and multiple fibroblast cultures derived from different individuals.

In a prior report, CAF was found to promote, rather than inhibit, the growth of MCF10A cells in a similar 3D co-culture system [22]. One of several possible explanations for this discrepancy between the prior result and the present result is a difference in E:F. Shekhar and colleagues used an E:F of 1:1 rather than the E:F of 2:1 we initially used [22]. The number of fibroblasts has been shown to have an effect on the response of epithelial cells [7,9,14]. We therefore repeated the 3D co-cultures of MCF10A cells using NAF-2 and CAF-1 with increasing numbers of fibroblasts (i.e. a decreasing E:F) (Fig. 6). BrdU labeling was assessed by immunocytochemistry of histologic sections of 3D cultures.

As previously, NAF-2 at an E:F of 2:1 suppressed proliferation of MCF10A cells. However, with increasing numbers of NAF-2, this suppression effect was gradually weakened ($P = 0.043$). Although we found no significant difference in the suppressive effect of NAF-2 in an E:F of 2:1 versus an E:F of 1:1 or of 1:2, there was a significantly greater rate of proliferation of MCF10A cells with NAF-2 in an E:F of 1:3 compared with in an E:F of 2:1 ($P = 0.028$). More importantly, CAF-1 at an E:F of 1:1 did not significantly suppress proliferation, whereas our original ratio of 2:1 did ($P = 0.025$). CAF-1 at an E:F of 1:2 also conferred a higher rate of proliferation of MCF10A cells than the E:F of 2:1, but this did not reach statistical significance ($P = 0.054$). At an E:F