

Fig. 3. Cancer-related (A) and disease-free (B) survival curves with reference to loss of claudin-1 in central parts of the tumors. Cancer-related (C) and disease-free (D) survival curves with reference to loss of claudin-1 at invasive fronts of the tumors. Cancer-related (E) and disease-free (F) survival curves with reference to loss of claudin-4 in central parts. Cancer-related (G) and disease-free (H) survival curves with reference to loss of claudin-4 at invasive fronts. Cancer-related (I) and disease-free (J) survival curves with reference to loss of E-cadherin in central parts. Cancer-related (K) and disease-free (L) survival curves with reference to loss of E-cadherin at invasive fronts. Cancer-related (M) and disease-free (N) survival curves with reference to loss of membranous  $\beta$ -catenin in central parts. Cancer-related (O) and disease-free (P) survival curves with reference to loss of membranous  $\beta$ -catenin at invasive fronts. Cancer-related (Q) and disease-free (R) survival curves with reference to aberrant nuclear  $\beta$ -catenin expression in tumor samples.

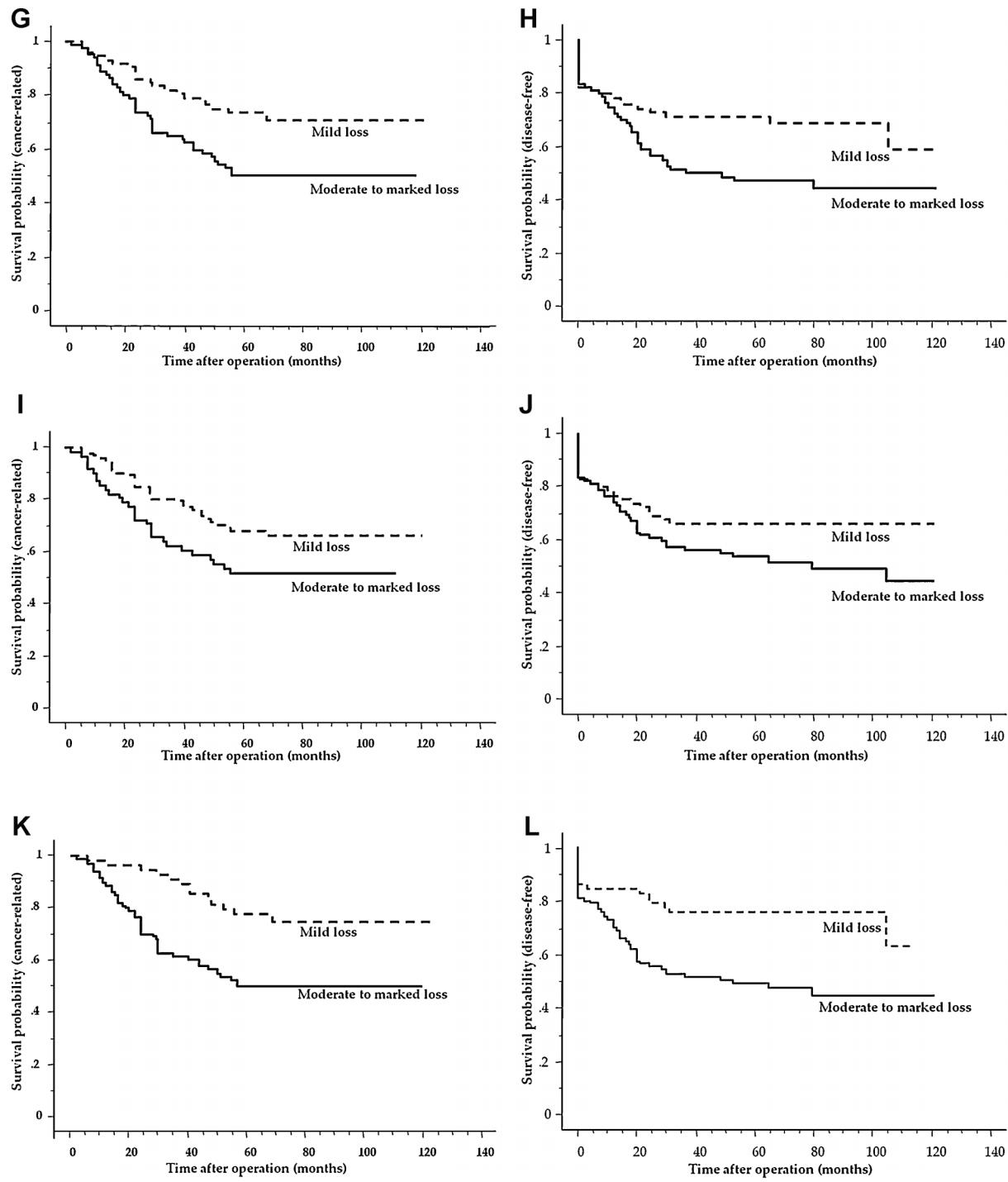


Fig. 3. (Continued)

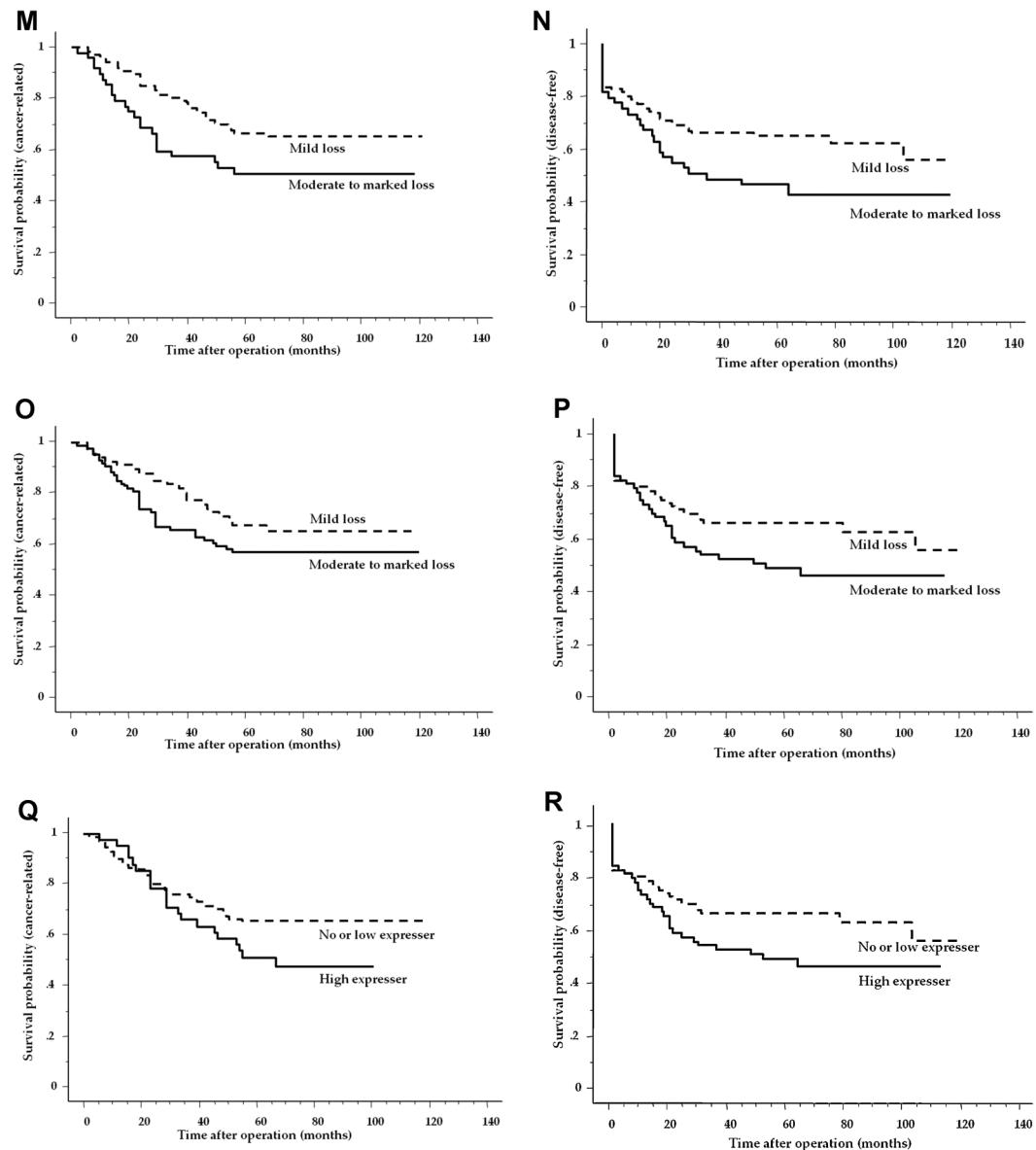


Fig. 3. (Continued)

TABLE VII. Multivariate Analysis of Variables for Cancer-Related and Disease-Free Survival

Variable	Cancer-related			Disease-free		
	HR	95% CI	P-value	HR	95% CI	P-value
E-cadherin expression						
Invasive front						
Mild loss	1			1		
Moderate to marked loss	3.423	1.734–6.756	<0.001	2.066	1.158–3.686	0.014
Membranous $\beta$ -catenin expression					NE	
Invasive front						
Mild loss	1					
Moderate to marked loss	1.798	1.006–3.213	0.048			
TNM stage						
II	1		<0.001	1		<0.001
III	3.571	1.652–7.717	0.001	3.512	1.788–6.897	<0.001
IV	46.18	19.47–109.5	<0.001	269.9	34.36–21.21 $\times 10^2$	<0.001

HR, hazard ratio; CI, confidence interval; NE, not examined because of no significant difference by univariate analysis.

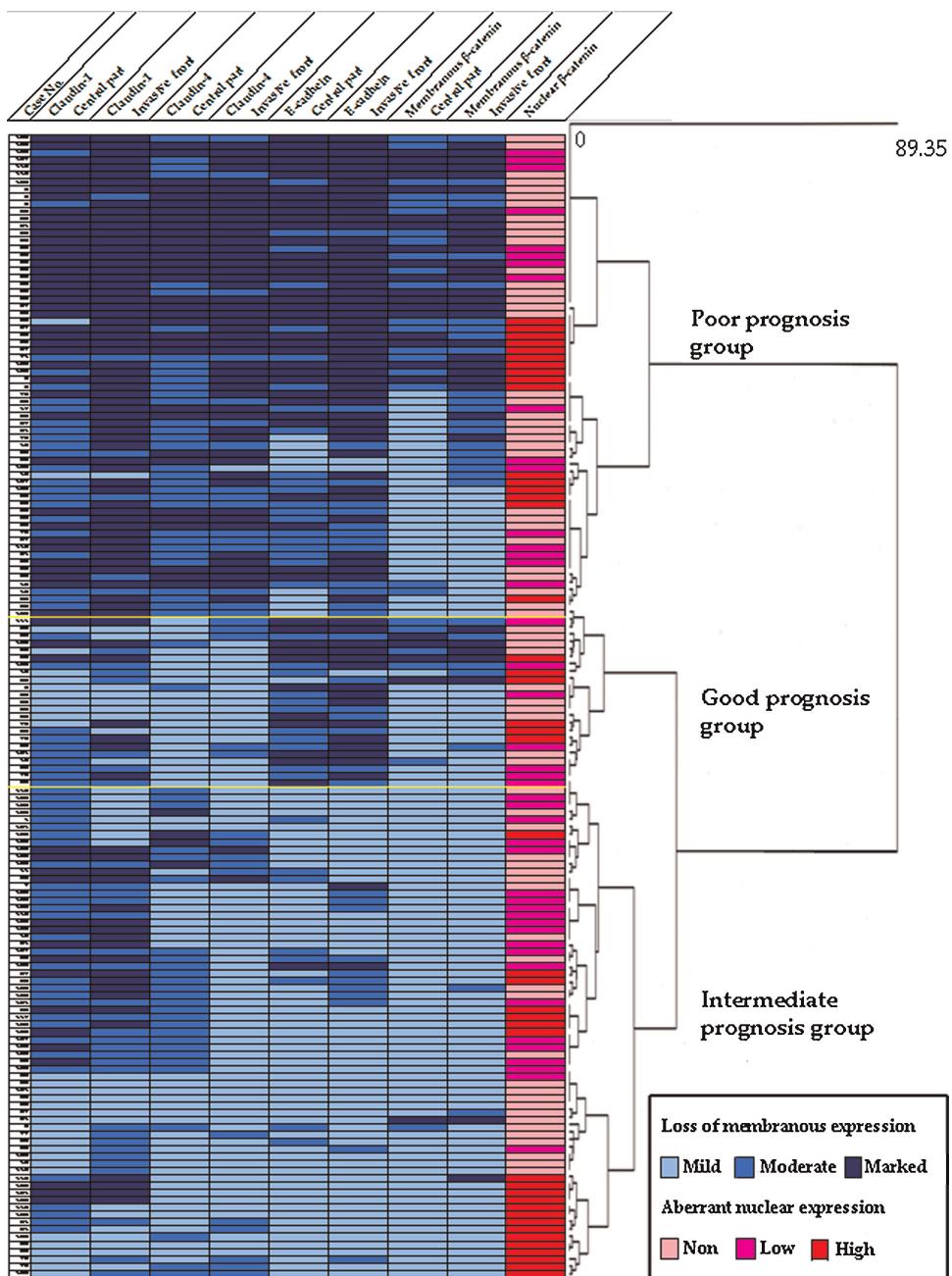


Fig. 4. Hierarchical cluster analysis of marker profiles in colorectal cancers. **Left**, a graphical representation of immunoreactivity; **right**, dendrogram produced by hierarchical cluster analysis.

earlier reported in stage II colon cancer with recurrence and poor survival [10]. Similar to claudin-1, reduced expression of claudin-4 at invasive fronts was shown to be significantly linked with poor tumor differentiation, deeper invasion, infiltrating growth pattern, lymphovascular invasion, and metastasis [12]. In addition, low-mRNA levels of claudin-1 were reported to be associated with poor tumor differentiation [14].

Our observations confirmed that in some of the cases studied claudin-1 and -4 were anomalously higher in small area of the tumor than in the

normal mucosa. However, their overexpressions were not significantly associated with any clinicopathological variables or prognosis (data not shown). Other researchers have described that claudin-1 or -4 proteins and mRNA levels were upregulated in colon cancer [11,13–15,18]. In vitro, overexpression of claudin-1 and -4 specifically stimulates invasive activity of colon cancer cells with activation of matrix metalloproteases [11,19]. Since such claudins are crucial components of tight junctions, alteration in their expression may affect cell proliferation, motility, and invasiveness in cancer cells in vitro, and in vivo loss of their



