Supplementary Table 1. The primers used for RT-PCR of nAChR from MSC are shown where "f" refers to the forward and "r" to the reverse primers

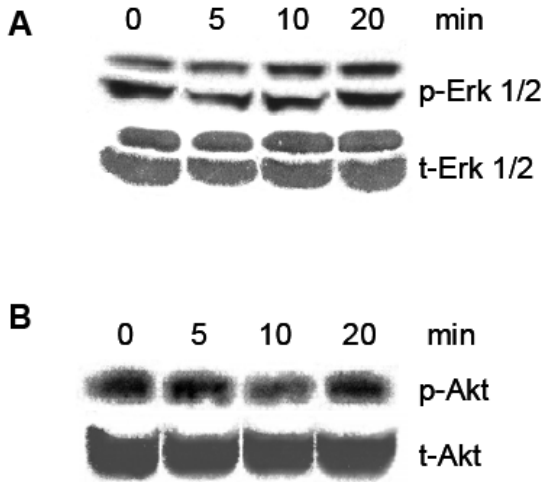
nAChR subunit	Primer sequence	Product size
α1	f: 5'-C GAC ACT ATC CCA AAT ATC ATG-3' r: 5'-TTG TTA GAC TCC TGG TCT GTC-3'	221 bp
α2	f: 5'-CTA CGC CTG AAG CTC AGC C-3' r: 5'-TCC TTC ACC GAA GAG TCA GC-3'	300 bp
α3	f: 5'-AC GAG GGC AAC GCT CAG AAG-3' r: 5'-CTC TTT GGC TTC ATT TTG TGC –3'	305 bp
α4	f: 5'-ATC GAG TCC ATG CAT AAG ATG-3' r: 5'-ACC GAG AAG TCT GTG TCT TC-3'	644 bp
α5	f: 5'-TT CAC ACG CTT CCC AAA CTG C-3' r: 5'-ATC ATT TTC CTT CAT GAT GTG TG-3'	169 bp
α6	f: 5'-TGG CCT CTG GAC AAG ACA AG-3' r: 5'-AGT CAT CTT CTA CCT CCT TGG-3'	301 bp
α7	f: 5'-CG TGG TTC CTG CGA ATG AAG-3' r: 5'-AAG CGG TTG GCA ATG TAG CG-3'	322 bp
α9	f: 5'-CTG AAA TAC ATG TCC AGG GTC-3' r: 5'-GTG GCC TTG TGG TCT TTG AG-3'	302 bp
α10	f: 5'-TG CTG GGA CAC CTG GCA CG-3' r: 5'-TCA TGG CAG CGC TGG GCA G-3'	234 bp
β1	f: 5'-A CCC GAG AGA GAC CTG ATG C-3' r: 5'-TTC AGC GCA TCG TGG TCC TC-3'	282 bp
β2	f: 5'-AG CAG CCA CGC CAT CAT TGC-3' r: 5'-CTC TGG TCA TCG TCC T CG C –3'	292 bp
β3	f: 5'-CTT TGC ATG AAA GAT CAT GTG G-3' r: 5'-TGT TCT TTC TTC ACA TGT CTC G-3'	197 bp
β4	f: 5'-TC TTC ATG AAG CGC CCT GGC-3' r: 5'-TCT CTG GTC TTC ATC GTC ATT C-3'	306 bp

Supplementary Table 2. Summary of nAChR sub-unit expression in comparison with the findings of Hoogduijn et al., 2008

nAChR subunit	Schraufstatter et al. RT-PCR	Schraufstatter et al. Western & IH	Hoogduijn et al. RT-PCR	Hoogduijn et al. FACS
α1	+			
α2	+			
α3	+	-	+	+
α4	+	-		
α5	+		+	+
α6	-			
α7	+	+	+	+
α9	+		-	
α10	-		-	
β1	-			
β2	+	+	-	
β3	+			
β4	+	+	-	

bungarotoxin suggesting a role for α7 subunits of nAChR in mediating these effects of nicotine. Unfortunately it was not feasible to block the effects of nicotine on hMSCs migration in vivo due to toxic side effects of α-bungarotoxin (not shown).

There are contradictory reports regarding the effects of nicotine on cell migration. While some groups reported that nicotine stimulates cell migration (Totti, McCusker et al. 1984; Nowak, Ruta et al. 1990; Li, Zhao et al. 2004; Di Luozzo, Pradhan et al. 2005), others demonstrated that nicotine is an inhibitory factor (Bridges, Kraal et al. 1977; Giannopoulou, Geinoz et al. 1999; Serobyan, Schraufstatter et al. 2005). We and others have previously published that nicotine induces signal transduction in non-neural cells (Kihara, Shimohama et al. 2001; Serobyan, Schraufstatter et al. 2005; Hoogduijn, Cheng et al. 2008), activation of which can be associated with increased cell motility. However, the signaling mechanisms activated might depend on the specific cell types and the diverse experimental conditions that were used in these different studies. For example, we detected Erk1/2 phosphorylation in endothelial cells (Serobyan, Schraufstatter et al. 2005), but not in hMSC (Supplementary Figure 1).



Supplemental Figure 1. The effect of nicotine on signal transduction in hMSCs. Human MSCs were cultured in 6-well plates will 60-70% confluence and treated with 10-7M nicotine for various periods of time as indicated. Phosphorylation of Erk 1/2 (A) and Akt (B) was measured by Western blot. Loading of the samples was controlled by measuring the total amount of Erk 1/2 and Akt.

Furthermore, the effect of nicotine on cells exposed to chemotactic factors might be different from that on serum-starved cells usually used for studying signal transduction. In addition, the effects of nicotine on cell migration may not necessarily be associated with signaling, but could be the result of a detrimental effect of nicotine on the expression of receptors for chemotactic factors, which was previously shown for CXCR4 (Serobyan, Jagannathan et al. 2007). Thus, further studies on the molecular mechanisms that mediate the effect of nicotine on cell migration are needed since nAChRs may represent an attractive therapeutic target for regulating the migration of non-neural cells.

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